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Volume 41

Edited by
G. C. AINSWORTH and S. D. GARRETT

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G. C. AINSWORTH, B.Sc., Ph.D., F.L.S.
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C. J. HICKMAN
President, 1957

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PRESIDENTIAL ADDRESS

PHYTOPHTHORA—PLANT DESTROYER

By C. J. HICKMAN

One of the outstanding moments of my student days was the first occasion on which I saw zoospore formation and discharge. The fungus was a species of *Pythium*. I was excited and fascinated to watch the homogeneous protoplasm of the sporangium become transformed into a milling mass of zoospores. As the zoospores encysted and began to grow, so the seed of my interest in Phycomycetes took root. During my apprenticeship to plant pathology at Long Ashton Research Station I was able to cultivate it. There I saw a number of *Phytophthora* species and, soon afterwards, I became intimately acquainted with another one, *P. fragariae*. As a mycologist I have come to have a great affection for the genus; as a plant pathologist, great respect. These then are the reasons that have prompted me to make it the subject of this address.

The genus was established by de Bary (1876) with *P. infestans* as the type species. It was included by him in the family Peronosporaceae. Later it was transferred by Fitzpatrick (1923) to the Pythiaceae because of the indefinite character of the sporangiophore and the development of sporangia in succession. These and other characters of the genus have been well defined recently by Blackwell (1949) and by Middleton (1952).

In the vegetative condition the typically right-angled branching of the non-septate hyphae and the constriction of the branches at the point of origin may provide the first clue to identification of a *Phytophthora*. Asexual reproduction is by the formation of sporangia in succession on sympodially branched sporangiophores which are undifferentiated from vegetative hyphae, or, as in *P. infestans*, for example, bear nodal swellings. Each new sporangium-bearing branch arises just below the previously formed sporangium, or, after zoospore liberation, by internal proliferation. The sporangia are typically ovoid to obpyriform, papillate, in shape, or in some species more elongated or irregular. Within them zoospores are differentiated and are released following the dissolution of an apical area of the sporangium wall which may swell up to form a hardly perceptible, sessile, evanescent vesicle. Sexual reproduction is characterized by the occurrence, in the majority of species, of amphigynous antheridia. In some, *P. erythroseptica* for example, all the antheridia are amphigynous, in others such as *P. fragariae*, both amphigynous and paragynous antheridia occur, while in *P. primulae* and a few other species only paragynous antheridia have been observed.

Having recognized a fungus as a Phytophthora one is faced with the task of naming it. This is not always easy. Although keys to Argentine and British species have been published recently by Frezzi (1950) and by

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Waterhouse & Blackwell (1954), respectively, no comprehensive taxonomic treatment of the genus has appeared since the monograph by Tucker (1931) and the species key by Leonian (1934). Since then a number of species have been added to the list. For some of these detailed descriptions and cultures are available. Others, particularly those from the Far East, are not so well known. Some of the new names I believe should be relegated to synonymy. In addition, new combinations of old established species have been put forward as a result of detailed, comparative work (Thomas & Ramakrishnan, 1948). There is thus a great need for taxonomic revision of the genus. Fortunately we now have the compilation of diagnoses and illustrations published last year by Miss Waterhouse (1956). Let us hope that a new taxonomic monograph will follow before very long.

Following the first tremendous impact of *P. infestans* in these islands towards the middle of the last century, we are annually reminded, as are those in other potato growing areas of the world, of the economic significance of this species. However, *P. infestans* is only one of many species in the genus and I propose first, to discuss their distribution and host range.

DISTRIBUTION AND HOST RANGE

The over-riding factor determining the distribution of *Phytophthora* species is, of course, moisture supply which governs development and spread in the asexual stage. It may be atmospheric moisture or soil moisture or both, depending on the particular species. In addition, we can observe with Tucker (1931, 1933) a correlation between distribution and temperature. Thus, for example, *P. palmivora*, *P. parasitica* and *P. citrophthora* are characteristic species of tropical and subtropical regions, whilst *P. cactorum*, *P. infestans*, *P. fragariae*, *P. phaseoli*, *P. cryptogea*, *P. porri*, *P. syringae*, *P. richardiae*, *P. cambivora*, *P. megasperma* and *P. erythroseptica* are well-known species from temperate regions. This climatic distribution of species is not hard and fast, however. For example, *P. hibernalis*, like *P. citrophthora*, attacks citrus in parts of Australia and California, but is active only in the cool winter months. *P. parasitica* is common in temperate regions where temperatures are high, as in greenhouses, and *P. cinnamomi* is reported from both temperate and tropical areas.

Considered as a whole, *Phytophthora* species are catholic in their parasitism, attacking a great range of plants, trees, shrubs, herbaceous plants, from many families of flowering plants and conifers. Amongst their hosts are a large number of important crop plants though, fortunately, cereals do not figure conspicuously amongst them. Considered individually, some Phytophthoras attack many different kinds of plant, while at the other extreme are those which are confined in their parasitism to a single family, genus or species. Outstanding in the former group are *P. cactorum*, *P. parasitica*, *P. palmivora* and *P. cinnamomi*. Excluding records of pathogenicity obtained by artificial inoculation, *P. cactorum* has been recorded on members of at least eighty-three genera distributed among forty-four families, including many ornamental plants, shrubs, fruit and forest trees and causing a variety of diseases, damping-off of seedlings, root rots, collar rots, trunk cankers, leaf and fruit rots. *P. parasitica*, commonly found in this country as a cause of buck-eye rot of tomato fruits, occurs on members of seventy-two genera in forty-two families of flowering plants. Included amongst its common hosts are tomato, tobacco, egg plant, cotton, citrus species, pineapple, various cucurbits, castor oil plant (on which it was first recorded), rhubarb, cinchona, betel pepper, as well as many ornamental plants. *P. palmivora* parasitizes plants of fifty-one genera in twenty-nine families of flowering plants. Common host plants are cocoa, rubber, various palms, cotton, citrus, pawpaw, pineapple, pepper, bread-fruit, jak, mango, guava, cinchona, and a number of orchids. *P. cinnamomi* is largely confined to woody hosts, trees and shrubs (Thorn & Zentmyer, 1954), causing root and collar rots. Described by Crandall (1950) as 'one of the world's major plant killers' it is very important, with *P. cambivora*, in causing ink disease of chestnuts in Europe and the U.S.A.; it also attacks other forest trees, beech, oak, walnut and conifers, both in the nursery and in the forest. It is a serious and widespread pathogen of avocado in various parts of the world, and other major hosts are cinchona, pineapple, pawpaw, camellia, cultivated heaths and rhododendrons.

Amongst species with narrower host ranges, *P. cambivora* was the first one to be recognized as a cause of ink disease of chestnuts, though it is now thought (Crandall, 1950) that *P. cinnamomi* is the more common cause. Like *P. cinnamomi*, *P. cambivora* is recorded most frequently from woody hosts, chiefly forest trees in temperate regions. *P. syringae* attacks leaves and twigs of lilac. It has also been shown responsible for a root rot of beech, collar rot and branch die-back of peach and apricot, fruit rot of apple and pear and gummosis and fruit rot of citrus. Most important as a causal organism of this citrus disease complex is *P. citrophthora*, found in citrus orchards the world over. *P. cryptogea* has been recorded on plants in twenty-five genera (including nine genera of the Compositae) in eleven families of flowering plants. It is commonly encountered as the cause of damping-off and foot rot of tomatoes and other herbaceous plants in the greenhouse, though it has also been reported causing serious root rot diseases of field crops (Erwin, 1956; Simmonds, 1956). *P. megasperma* has been isolated from members of fourteen genera in nine families of dicotyledons, mostly herbaceous plants and *P. erythroseptica*, known from many parts of the world as the cause of pink rot of potato tubers, is also recorded from tulip, onion, tomato, calla lily and sugar cane.

Having regard to the wide host range of many Phytophthoras it is not surprising to find that certain plants are attacked by several species, though the symptoms produced by each may be identical. Shanking of onions and gummosis and fruit rot of citrus provide good illustrations of this situation. Four species, *P. cactorum*, *P. cryptogea*, *P. erythroseptica* and *P. porri* are associated with the onion disease, while from citrus fruits suffering from brown rot not only *P. citrophthora* but also *P. cactorum*, *P. hibernalis*, *P. palmivora*, *P. parasitica* and *P. syringae* have been isolated.

In great contrast to such omnivorous species as *P. cactorum*, a number of others are very restricted in host range. Of these, *P. infestans* is confined to members of the Solanaceae. More limited still is *P. primulae*, occurring on primula and polyanthus whilst at the extreme of parasitic specialization are others pathogenic to a single plant species. These include *P. phaseoli* from lima bean, *P. richardiae* from arum lily, *P. inflata* from elm, *P. quininea* from cinchona and, one of the most recent additions to the genus, *P. ilicis*, from holly. It is possible that some of these last-named species may ultimately prove to be less specialized than is at present indicated. Thirteen years after it was described from leeks *P. porri* was isolated from onion (Hickman, 1944; Tomlinson, 1951) and, a few years later, from Campanula (Legge, 1951). Similarly, *P. fragariae*, until recently found in the field only on strawberry, and proved pathogenic only to species of *Fragaria* (Bain & Demaree, 1945), has now (McKeen, 1956) been identified on loganberry. On the other hand, *Phytophthora phaseoli* described as long ago as 1889, is still recorded only from lima bean (Cox & Hyre, 1951).

Not infrequently with diseases now known to be caused by species of *Phytophthora*, the pathogen has remained unrecognized for some considerable time. Some species are inconspicuous on the host, difficult to isolate and are accompanied, or closely followed, by other, more easily isolated organisms. Sometimes these latter have been mistakenly identified as primary pathogens. Thus although chestnut root rot was observed in the U.S.A. in 1912 (Crandall, Gravatt & Ryan, 1945), it was not until 1931 that, with an improved technique, *P. cinnamomi* was isolated, *Armillaria mellea* meanwhile having been suggested as the cause of the trouble. The difficulty of isolating this Phytophthora is indicated by the fact that in a survey a few years later only just over 4% of attempts to isolate from freshly dug infected roots were successful. A few species, including *P. verrucosa* which was described in 1940 (Foister, 1940), have not yet been grown in pure culture. Added to the difficulty of getting it to grow out from the host, failure to appreciate that a Phytophthora is active only for a certain period of the year may further hinder its isolation. Not only is *Phytophthora fragariae* closely followed by fast growing secondary organisms, making it sometimes difficult to isolate on that account, but it is active only from autumn until spring and cannot be isolated at other times of the year (Hickman, 1940). In fact, following secondary breakdown of infected roots, it is virtually impossible to find any evidence of the fungus whatsoever from June, when above ground symptoms are most conspicuous, until infection of new roots occurs with the onset of autumn rains. *P. ilicis* behaves similarly (Buddenhagen & Young, 1957). It attacks leaves and twigs during winter and spring and can readily be isolated during this period. For the rest of the year the pathogen is inactive and isolations from killed tissue yield secondary organisms, two of which, *Boydia insculpta* and *Phomopsis crustosa*, have previously been reported as the cause of the disease. Oospores of *Phytophthora ilicis* were found in preserved herbarium material collected twenty years before the fungus was recognized in the field.

One hitherto unknown *Phytophthora* disease, described recently, owes its recognition to the fact that by chance the precise conditions for its optimum development were provided while the host plants were under close observation for other reasons. In a study of the effects on peas of *Fusarium solani* and the eelworm *Heterodera gottingiana*, Bywater (1956) grew plants in a compost of which one of the ingredients was soil contaminated with these two organisms, collected from a Worcestershire market garden. It was with mixed feelings that Dr Bywater watched her plants collapse with a severe root rot quite unlike that due to *Fusarium* or *Heterodera*. Observing in the rotted roots oospores contained in oogonia bearing amphigynous antheridia, she isolated the fungus and successfully reproduced the symptoms by inoculation with a zoospore suspension. The fungus, a Phytophthora, could not be identified with any named species and Dr Bywater has proposed for it the name *Phytophthora pisi*. Since the disease did not appear amongst plants growing in compost without field soil it was clear that it had been introduced in the field soil itself. It remains to be seen whether the disease occurs in field crops.

SURVIVAL

One species of *Phytophthora*, *P. inflata*, causing pit canker of elm (Caroselli & Tucker, 1949), appears to grow continuously in its host from year to year, but for the other species there is usually an annual period when, through the death of the host, or the onset of unfavourable weather conditions, development ceases. For many species this is the winter but for others, for example, *P. hibernalis*, *P. fragariae* and *P. ilicis*, it is the summer. Some species survive this period in living host tissue. Studies of this aspect of *Phytophthora* biology received their initial stimulus when de Bary (1863) demonstrated the growth of *P. infestans* from diseased potato tubers into young shoots. This has since been observed on a number of occasions, although its precise importance in relation to the initiation of blight outbreaks is still under investigation (Hirst, 1955; Van der Zaag, 1956). In addition to providing a means of overwintering, the mycelium of *P. infestans* has been shown recently to survive for several weeks of hot, dry, summer weather in potato stems (Clayson & Robertson, 1956). Other examples of survival of mycelium in living host tissue include *P. colocasiae* in the corms of its host, taro (Butler & Kulkarni, 1913), *P. palmivora* in the cankered 'cushions', from which the flowers and pods arise, on the trunks of cocoa trees (Dade, 1928, 1929), and on rubber trees, at the margins of lesions on branches and fruit stalks (McRae, 1918). One species, *P. capsici* (Leonian, 1922), attacking chile pepper, is seed-borne and another, *P. phaseoli* (Cox & Hyre, 1951), is suspected of overwintering in this way as well as in crop residues.

More generally important as a reservoir of *Phytophthora* infection is the soil. Many species, *P. cinnamomi*, *P. fragariae*, for example, attack their hosts through the roots or other underground organs. Alternatively, as in *P. phaseoli* and *P. porri*, infected aerial parts fall to the ground or are ploughed in after the crop has been taken. However introduced, the pathogen is ultimately released into the soil on the decay of the infected tissues. The capacity of *Phytophthora* species to survive in soil, with its obvious and important bearing on the development of effective control measures, has been the subject of many investigations. These have not only demonstrated the presence of *Phytophthora* in soil but also their survival for longer or shorter periods in the absence of host plants (Anderson, 1951; Baines, 1939; Campbell, 1951; Cox & Hyre, 1951; Critopoulos, 1955;

Fraser, 1949; Grente & Solignat, 1952; Ogilvie, 1931; Orellana, 1954*a*; Smith & Smith, 1906; Smith, 1955; Thorold, 1955; Valleau, 1951; Wright, 1930; Zentmyer & Munnecke, 1952; Zentmyer & Popenoe, 1951). What is not clear, however, is the relative importance of mycelium and oospores in this survival. Do these fungi embark on a saprophytic phase, actively growing, on entry into the soil (Thung, 1938; Fraser, 1949), or do they merely lie dormant, like members of the Peronosporaceae, for example, in the form of oospores or other functionally homologous structures (Blackwell & Waterhouse, 1930) such as chlamydospores (Cairns & Muskett, 1933; Clinton, 1906; Hickman, 1940)?

In a recent, valuable contribution to this aspect of *Phytophthora* biology, Legge (1952, 1953) emphasized the fact that no conclusive answer to this question can be provided by indirect methods of observation, relying on the isolation of the fungus from baits of host tissue, or on its identification in plants grown in the soil, such as have been generally used. She developed a simple but ingenious method of studying, directly, the behaviour of *Phytophthora* in the soil. This involved the burial of glass fibre tape interlaced with hyphae bearing sporangia and oospores. By examining tapes at intervals she was able to follow what happened to the mycelium and reproductive organs.

Studying *P. cactorum* and *P. megasperma* in this way she found, as has been demonstrated for other species (de Bruyn, 1922; Foister, 1931), that in sterile soil hyphae continued to grow. In non-sterile soil, however, the hyphae, antheridia and oogonia decomposed, in that order, leaving the oospores which germinated, some after burial for a week, some after a year. While the proportion that germinated after a few days was very small, it was nevertheless an interesting feature of these experiments, for previous work with *P. cactorum* (Blackwell, 1943*a*, *b*) has shown that in agar culture oospores pass through a dormant period lasting several months before they germinate. Here may be mentioned too the recent demonstration by Gregg (1957) that, after passage through the alimentary canal of garden snails, oospores of *P. erythroseptica* and *P. cactorum* were also stimulated to germinate within a few days. Whether this stimulation is due to the activity of cellulase, which is secreted by snails in large amounts, or to the action of micro-organisms in the snail intestine is not yet known.

Germination of the oospores from the glass fibre tapes took place by the development of germ tubes which quickly gave rise to sporangia. There was no evidence of growth of hyphae beyond that required for sporangium production. Pointing out that outbreaks of soil-borne *Phytophthora* diseases usually coincide with wet conditions, conditions which favour formation of sporangia, Dr Legge suggests that under natural conditions these arise from germinating oospores, or similar perennating bodies, and concludes that perennation in the form of saprophytically growing mycelium is unlikely.

In support of this conclusion is the fact that no one has succeeded in isolating a Phytophthora directly from soil (Chesters, 1949), whereas well-known saprophytes such as *Pythium* are frequently isolated. Further, there is the fact that some *Phytophthora* diseases (e.g. Valleau, 1951), can be controlled by a sufficiently long crop rotation. Quoting Dr Legge: 'This fits in well with the idea of the longevity of the oospores and their gradual disappearance from the soil by fractional germination of their numbers; whereas if *Phytophthora* is well able to live saprophytically in the soil there seems no sound reason why it should die out at all.'

It is difficult to explain the persistence of *P. cactorum* in air dry soil, reported by Mes (1934), on any other basis than oospore survival but, on the other hand, the results of some experiments with *P. fragariae* do suggest that, under certain conditions, this fungus may survive in the soil, if not develop saprophytically, in the form of mycelium. Inoculum was added to soil as zoospores and as mycelium in 4 mm. diameter disks of bean agar, an agar on which oospores have never been observed. Using as a criterion of viability infection of strawberry runners planted in the soils at intervals after addition of inoculum, with frequent watering to encourage the disease, there was little infection in the zoospore inoculum series, even in plants potted up immediately, and none at all when the interval after addition of inoculum was three or more weeks. Where mycelium in agar was added, the amount of infection, initially high, decreased during the first four months to a very low level, at which it remained over the next fifteen months. No infection was obtained in still later tests. Thus, after introduction as mycelium, the fungus lingered on in non-sterile soil for just over a year and a half, a surprising result. It was not possible in this experiment to compare survival of the fungus after addition of roots containing oospores but in the field, after natural addition of this sort of inoculum, *P. fragariae*, remains viable for a much longer period, at least thirteen years.

It is clear that there is need of much more research on this aspect of the biology of these very interesting fungi. This is further emphasized by the related observation of Miss Blackwell and her colleagues (Blackwell, Waterhouse & Thompson, 1941; Blackwell, 1944), that *Phytophthora* species may live saprophytically as water moulds.

Reverting for a moment to asexual reproduction in soil-borne species, there is no doubt that soil contains a factor or factors which, *in situ*, in a natural leachate such as pond water, or in a prepared leachate, stimulates sporangium production. Long ago it was observed (Smith & Smith, 1906) that mycelium of *P. citrophthora*, growing on the surface of the soil (from infected lemons), produced very numerous sporangia and 'made such ground highly infectious'. Describing this fungus as a soil inhabitant, Fraser (1949) made a similar observation as did Carne (1925) for *P. hibernalis*. Some years ago (Hickman, 1940) I observed sporangia of *P. fragariae* growing out from the tips of infected strawberry roots in wet soil and I have used non-sterile pond water (pH 6·8) very successfully (Hickman & Goode, 1953) for the production of sporangia in this and other *Phytophthora* species.

Several workers have reported the stimulatory effect of soil leachates on asexual reproduction (Bywater, 1956; Davies [*pers. comm.*]; Mehrlich, 1935; Tomlinson, 1952; Wills, 1954). Working recently with mycelium of *P. parasitica* var. *nicotianae*, from liquid culture, Wills concluded that sporulation is induced by the provision, in correct balance and concentration, of certain inorganic ions. The presence of salts as carbonates was particularly important. (Another mineral solution, Petri's solution (Petri, 1917) has, of course, been used with varying success, for many years.) We have found at Birmingham that some, but not all, isolates of *P. fragariae* are stimulated to sporulate from bean agar in Wills's solution, though not so well as in pond water. Mineral ions are apparently not alone in inducing sporulation in this fungus for Davies, examining the effects of the constituents of a greenhouse compost on sporangium development, found that some organic component of the peat fraction was the most active factor.

DISPERSAL

The dependence on high soil moisture of soil-borne *Phytophthora* species has not infrequently led to the conclusion that soil waterlogging is the primary cause of the disease (Fraser, 1949; Hickman, 1940). The importance of soil water in the biology of these fungi lies, not only in the fact that it stimulates asexual reproduction but also in that it is the agent of zoospore transport. Unaided, zoospore movement through soil is virtually nil (Hickman & English, 1951*a*) but, borne by moving water draining through the soil after heavy rain, or following irrigation, or in streams, they may be carried some distance (Bewley & Buddin, 1921; Fraser, 1949; Lewcock, 1935; Nolla, 1928; Sarejanni, 1952; Thung, 1938; Tucker & Milbrath, 1942; Welsh, 1942; Zentmyer & Richards, 1952).

Although some of the species attacking the aerial parts of the host are also spread by zoospores, dispersed in drops of rain water as in *P. ilicis* (Buddenhagen & Young, 1957), the major role in their dissemination is played by the sporangium itself. While the sporangia of soil-borne Phytophthoras such as *P. fragariae* and *P. cinnamomi* remain attached to the sporangiophore, those of many species above ground, of *P. infestans*, *P. palmivora* and *P. phaseoli*, for example, are caducous and, after liberation, are borne aloft in large numbers as part of the air spora (Gregory & Hirst, 1957; Heuberger, Cox & Hyre, 1950; Hirst, 1953; Thorold, 1955). Each stage of the process of asexual reproduction is closely controlled by humidity and temperature. Beginning with the work of van Everdingen (1926) and later of Beaumont (1947), this has led in this country to one of the most fruitful lines of modern, co-operative, plant pathological research; namely, the development and use of methods for forecasting outbreaks of potato blight, which, in combination with field methods for visually estimating blight losses now provides a sound basis for advice on the use and timing of fungicide applications (Large, 1955).

PHYSIOLOGIC SPECIALIZATION

I have talked in general terms about *Phytophthora* species, their distribution and host range, their survival and spread. In conclusion, I should like to refer briefly to physiologic specialization. Many papers have been published on physiologic specialization in *Phytophthora*. Almost all of these concern *P. infestans* where the phenomenon has been closely studied for a quarter of a century following the introduction of wild *Solanum* species, in particular *S. demissum*, as breeding parents. Amongst other species of *Phytophthora*, *P. citrophthora* (Fraser, 1949) is stated to be 'a simple species without specialised strains', while there are scattered references to pathogenic variation in *P. cactorum* (Baines, 1939; Smith, 1955, Stamps, 1953*a*; Tucker, 1931), *P. cinnamomi* (Mehrlich, 1936; White, 1937; Torgeson, 1954), *P. cryptogea* (Erwin, 1954), *P. erythroseptica* (Tompkins & Tucker, 1947), *P. palmivora* (Gadd, 1924; Orellana, 1954*b*; Tucker, 1931) and *P. parasitica* (Apple, 1954, 1955; Leonian, 1926; Tucker, 1931).

From 1948 onwards there has been growing realization of its importance in relation to the breeding of strawberries resistant to *P. fragariae*. Following the devastation of the strawberry-growing industry by this fungus in south-west Scotland during 1921–30 and the recognition that control of the disease by cultural or chemical means was an impossibility, systematic breeding for resistance was begun in 1933 by Reid at the West of Scotland Agricultural College. The first suggestion of the existence of physiologic specialization in *P. fragariae* was made fifteen years later when Reid (1948) observed that after remaining resistant for a number of years, seedling cultivars which he had raised, became severely infected in certain areas after they had been released for commercial production. Extensive tests using as inoculum infected roots from a number of sources led him (Reid, 1952) to conclude that a number of strains of *P. fragariae* exist. Further evidence of physiologic specialization in this species has since been obtained, both in this country and in America (Hickman & English, 1951*b*; Jeffers & Scott, 1952; Montgomerie, 1956) and at Birmingham we have confirmed Reid's conclusion using pure cultures for tests with forty-nine isolates on six host differentials, which have led to the recognition of twelve races of the pathogen.

On the question of the origin of pathogenic variation in *Phytophthora* there is little precise information. Much of it no doubt arises as a result of sexual reproduction though this has yet to be unequivocally demonstrated. For *P. infestans* indirect evidence has come to light very recently in the discovery, in Mexico (Niederhauser, 1956; Gallegly & Galindo, 1957; Smoot, Gough & Gallegly, 1957), of two sexually compatible strains, forming oospores when paired together in leaves or in culture, together with a range of physiologic races unknown in other areas such as Europe. The curious fact has emerged that blight races from the British Isles, Western Europe, Africa and North America belong to the same group, and are therefore sexually incompatible, forming oospores only when paired with one of the Mexican strains. Heterothallism has been reported for a number of other species in the genus. Attention has been also drawn to the possibility of interspecific mating (Cohen, 1950; Stamps, 1953*b*). An obstacle to the study of the products of sexual reproduction in *Phytophthora* has been the difficulty of readily inducing oospore germination. However, it is not insuperable for Barrett (1948) has studied segregation for mating type amongst the progenies of oospores of *P. drechsleri*. The development of work such as Dr Gregg (1957) has begun on the stimulation of oospore germination in *P. erythroseptica* may prove to be invaluable in this connexion.

Changes of pathogenicity ascribed to mutation have also been described, in *P. infestans* (Wriedt, 1955) and in *P. parasitica* (Leonian, 1926; Apple, 1955) for example, while in *P. cactorum* Stamps (1953*a*) found a type of variability that can perhaps better be explained in terms of cytoplasmic change. It is possible too that the adaptive changes in the pathogenicity of strains of *P. infestans* which have been observed on several occasions (de Bruyn, 1951; Mills, 1940; Reddick & Mills, 1938) may be of this nature. These are problems for the future. However, the rapid and spectacular progress that is now being made in the general field of variation in fungi suggests that answers will not long be delayed.

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THE USE OF CELLOPHANE IN GROWTH STUDIES ON SOIL FUNGI

By A. KERR

Waite Agricultural Research Institute, Adelaide, South Australia

A cellophane disk technique is described and has been used to demonstrate a stimulation of *Pellicularia praticola* by fresh grass cuttings. The technique has serious limitations, however, because the behaviour of a fungus between sheets of cellophane may be very different from that in the surrounding substrate.

The two methods generally used for studying the growth of fungi in soil are the Rossi-Cholodny slide and the soil tube. Techniques using glass in soil have been criticized by Dobbs & Hinson (1953), who state that water droplets condense on the glass, and the behaviour of fungi in these droplets is different from their behaviour in soil. They described a new method using cellophane bags, sprayed or dusted with fungus spores and half buried in soil, and by this means these authors demonstrated a widespread fungistasis in soils.

In a study of 'Brown Patch' disease of lawns, the saprophytic growth of the causal fungus, *Pellicularia praticola* (Kotila) Flentje, was observed in various substrates using a slight modification of the Dobbs & Hinson technique. Sheets of cellophane (25 μ thick) were cut into disks 12 cm. in diam., boiled in water to remove any coating materials, dried, and then joined in pairs by sealing the periphery with Perspex dissolved in chloroform, leaving 1 in. of the circumference unsealed. The paired disks were left for 24 hr. to allow the chloroform to evaporate, and were then inoculated with a 4 mm. plug of inoculum placed in the centre between the cellophane sheets. To minimize the nutrient added, the inoculum was taken from the growing edge of a distilled-water-agar culture, so that growth was dependent on nutrients diffusing through the cellophane from the substrate. After inoculation the 1 in. gap previously left in the circumference was sealed with adhesive, and the cellophane disks were placed in 14 cm. Petri dishes to which various substrates had been added. Before measuring fungal growth, the outside of the cellophane was wiped carefully with moist cotton-wool to remove any adhering substrate. The diameter of the colony was measured by direct observation through the cellophane and then a radial strip 1 cm. wide was cut from each disk, and stained with cotton-blue in lactophenol, to obtain details of density and form of fungal growth.

Growth of *P. praticola* was studied in washed, sterilized sand, unsterilized soil, sterilized soil and fresh grass cuttings. Disks were removed for examination after 2, 4 and 6 days and there were six replicates of each treatment (Table 1). Growth in washed sterilized sand represents the capacity of the fungus to grow from the inoculum plug with little or no influence of nutrients or antibiotics from the surrounding substrate. *P. praticola* grew extensively though sparsely, and apparently is able to spread considerably with a minimum of food.

Table 1. *Growth of* Pellicularia praticola *on cellophane disks in four substrates*

(Mean diameter of six colonies (mm.).)

	Substrate			
Days after inoculation	Sand	Soil	Sterilized soil	Grass
2	25	17	20	26
4	44	19	31	57
6	73	20	33	58

In sterilized soil growth was only slightly greater than in unsterilized soil. This result was unexpected, because when soil is sterilized there is a considerable release of nutrients and the micro-organisms capable of using them are destroyed; one would expect a fast-growing fungus such as *P. praticola* to grow more vigorously in sterilized than in unsterilized soil. Even in the latter, growth was surprisingly limited in view of Blair's (1943) conclusion that *P. filamentosa*, a fungus very similar to *P. praticola*, was capable of indefinite growth through soil. It is possible that the cellophane disks prevented the fungus utilizing the available food material in the soil. This was investigated in an experiment involving two soil treatments (sterilized and unsterilized) and two points of inoculation. The cellophane disks were inoculated as before, but in addition a plug of inoculum was placed on the outside of each disk. The disks were carefully removed from the soil after 2, 4 and 6 days, and the growth of *P. praticola* hyphae on the outside of the disks was observed under a dissecting microscope. Growth in unsterilized soil was very sparse and it was impossible to measure its extent on the outside of the cellophane, but in sterilized soil there was vigorous growth of the fungus (Table 2). As in the previous experiment, growth of *P. praticola* between the disks of cellophane was very limited and was much less than in the surrounding soil: the cellophane probably prevented the fungus utilizing the available food by being impermeable either to certain nutrients from the substrate or to some enzymes from the fungus. Thus the behaviour of the fungus between sheets of cellophane was very different from that in the surrounding substrate. This has been demonstrated conclusively only for *P. praticola* in sterilized soil, but it is likely to apply to other fungi and other substrates.

Table 2. *Growth of* Pellicularia praticola *on the inside and outside surfaces of cellophane disks in sterilized soil*

(Mean diameter of six colonies (mm.).)

Days after inoculation	In disk	In soil
2	20	23
4	31	69
6	31	110

Fresh grass cuttings appeared to stimulate *P. praticola* for 4 days, growth being more extensive and much denser in this substrate than in sand, but after 4 days growth ceased, accompanied by a swelling and bursting of the hyphal tips. There was abundant growth of fungi, actinomycetes and bacteria on the outside of the cellophane disks, and growth of *P. praticola* might have been prevented either by the antagonistic action of these organisms or by the disappearance of the original stimulus. This was investigated by transferring disks from grass after 4 days to freshly cut grass, and by placing new disks in the grass cut 4 days previously; no further growth occurred in disks from either treatment, indicating that only freshly cut grass stimulates *P. praticola*, and that if the cellophane has been colonized previously by other micro-organisms, the stimulus is obscured.

Blair (1943) found that grass cuttings depressed the growth of *P. filamentosa*. It is likely that there is an initial stimulation of such 'sugar fungi' by the nutrients released from grass cuttings, but that this is followed rapidly by an inhibition, due to the increased population of other micro-organisms.

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STUDIES ON *LEVEILLULA TAURICA* (LÉV.) ARN. AND OTHER POWDERY MILDEWS

By M. A. NOUR

Faculty of Agriculture, Shambat, University of Khartoum, Sudan

(With 4 Text-figures)

The ability of conidia of *Leveillula taurica* from one host species to infect other hosts belonging to different families has been demonstrated in the Sudan. Several cultivars of Egyptian cotton (*Gossypium barbadense*) and of American cotton (*G. hirsutum*) became infected when artificially inoculated from diseased leaves of *Euphorbia heterophylla* (an ubiquitous weed in Northern Sudan) or *Faba bona*. Cross-inoculations in the reverse direction were equally successful. On the other hand, conidia from diseased leaves of *Abutilon figarianum* failed to cause infection of any other host. Conidial measurements made from different hosts collected at different localities showed that those obtained from *A. figarianum* were consistently smaller than the rest. It is believed, therefore, that this 'strain' must be a different variety or even species of *Leveillula*.

The washing of healthy leaves of *E. heterophylla*, after inoculation with conidia of *L. taurica* (at Shambat) had little or no effect if delayed for more than 3 hr. after inoculation. The treated plants, kept at 100% R.H., showed no reduction (or increase) in percentage infection when compared with treated plants in the 'free' atmosphere. Similar results were obtained on young barley leaves inoculated with conidia of *Erysiphe graminis* at Rothamsted Experimental Station.

Since penetration by the infection hypha of both parasites (and of the other species of the Erysiphaceae) occurs normally 24–48 hr. after inoculation, the resistance of the conidia to washing off the leaf surface must be due to 'physical' adhesion prior to penetration. The infection and development of the disease under constant saturation being quantitatively similar to that under normal climatic fluctuations tends to refute the opinion that powdery mildew infection and intensity are reduced by high humidities.

Despite the varying climatic conditions of the Sudan fresh conidia of *L. taurica* were able to germinate under a wide range of humidity (0–100%). Germination was best at 85–100% R.H. with long germ tubes and turgid conidia, but was poor at lower humidities (0–30%) with short germ tubes and shrunken conidia. Conidia submerged in water gave poor germination; better germination was obtained from floating spores. The effect of humidity and of water tested on conidia of *E. graminis* from barley (at Rothamsted), gave similar results except in that they were more or less as sensitive to humidities below 95% R.H. as the conidia of *E. cichoracearum*, *E. umbelliferarum* and *Sphaerotheca fuliginea* tested in the Sudan.

SPECIALIZATION OF *LEVEILLULA TAURICA* IN THE SUDAN

Powdery mildews are obligate, specialized parasites. Most of the work on their specialization was concerned with *Erysiphe graminis* DC. (Marchal, 1902; Salmon, 1905; Reed, 1909), and to a lesser extent other species of *Erysiphe*. *E. polygoni* DC., for example, was considered by Blumer (1933) as an aggregate species which he divided into several separate species. Other workers have found several physiologic races of *E. graminis tritici* and *E. graminis hordei* in nature; at least five races of the former and twelve of the latter are known to occur in North America (Mains & Dietz, 1930; Newton & Cherewick, 1947), and at least two races of *tritici* in New Zealand (Smith & Blair, 1950).

Tendencies towards a subspecialized habit by some mildews, however, have been observed. Thus, according to Salmon (1905), conidia of *E. graminis* from wheat can infect *Hordeum silvaticum*, and Hardison (1944) found that *Agropyron* spp. can be infected by conidia of *E. graminis* from wheat, barley and *Elymus* spp. Hammarlund (1945) reports the presence of highly polyphagous species in *Erysiphe*, e.g. conidia of *Oidium begoniae* (from *Begonia*) can infect sixty-two species belonging to eleven different families.

Under cool climatic conditions the powdery mildews commonly produce resistant cleistothecia containing ascospores which may remain viable through the unfavourable winter season, and cause primary infection with the onset of the spring, if the appropriate host is available. Perennation is also reported to occur by means of resistant or dormant mycelium of the fungus on the dead straw (Cherewick, 1944), in buds (Woodward, 1927) and in the seed (Uppal, Patel & Kamat, 1935). Powdery mildews may also persist throughout the unfavourable season on volunteer plants (Honecker, 1937; Gorlenko, 1940), or in the conidial state on young winter wheat (Turner, 1956).

In the tropics and subtropics, however, the powdery mildews rarely produce cleistothecia, and the occurrence of heat or drought resistant mycelium has not been demonstrated. The source of primary infection of some powdery mildews under extreme aridity and heat as in the Sudan is, therefore, not yet known. Many susceptible crops are grown during the winter season in the Sudan, when several powdery mildews appear and spread on them (Tarr, 1955). The crops are harvested in March or April and are sown again 6 or 7 months later. It may be argued that primary infection could be established by wind-blown conidia from farther north during the winter, as is probably the case for uredospores of *Puccinia graminis* Pers. in the Sudan and Egypt, and also, as reported from India for *P. graminis* uredospores (Mehta, 1931) and for *E. graminis* conidia (Mehta, 1930). However, as the conidia of powdery mildews are short-lived, their long-range dissemination would seem highly unlikely especially under Sudan conditions where conidia would have to be blown and remain viable over hundreds of miles, if the primary source of infection had to be from Egypt. This problem of the source of infection remains, therefore, one of the major ones in the ecology of these diseases.

Leveillula taurica (Lév.) Arn. is a parasite of a wide host range in the Sudan (Boughey, 1946; Tarr, 1955) and elsewhere (C.M.I. map no. 217). Very little work has been done on its nature and specialization since that of Salmon (1906), but Zwirn (1943) and Kamat & Patel (1948) found that *L. taurica* is highly specialized to within the bounds of a species of the host plant.

During a series of field tests on broad bean, it was observed that mildew infection appeared more widespread, where *Euphorbia heterophylla* (a common weed) was growing. This work was undertaken, therefore, to find out whether *L. taurica* can behave as a generalized parasite.

Experimental techniques

Cross-inoculations. Faba bona Medic. (broad bean) plants were grown in pots during July–September 1954 under shade in a cool 'Mashtal' constructed with bamboo sticks 1 in. apart. Being a delicate plant and grown out of season, death was frequent and the tests had to be repeated several times.

Five pots, containing four plants each, were placed in a different 'Mashtal' every time a test was made. One leaflet selected at random on each plant was marked with indian ink and inoculated with fresh conidia from diseased leaves of *E. heterophylla* L., and thus twenty leaflets were inoculated. Another twenty leaflets on another five pots were inoculated from diseased leaves of *A. figarianum* Webb., taking adequate precautions to prevent cross-contamination. Inoculation of the leaflets was effected by contact with the diseased area of a mildewed leaf. Infected leaves of *Aristolochia bracteata* Lam., *Gossypium hirsutum* L. and *G. barbadense* L. were also used to inoculate bean leaflets.

Where the disease was established on the bean leaflets, they were immediately removed and used in the same manner for inoculating: (*a*) healthy bean leaflets, and (*b*) healthy leaves of the host from which the original inoculum was obtained. The tests were repeated during July 1955–March 1956, on healthy leaves of both *F. bona* and *E. heterophylla*. The technique was modified, however, by restricting inoculation to one-half only of the leaf (or leaflet) on one side of the midrib, the other half being shielded by a folded paper with a clip for 24 hr. after inoculation.

The data given in Table 1 were obtained, therefore, from the 1955–6 tests, although there were no fundamental differences between the results from these and the 1954 results. They represent the means for each treatment combination. Tests were also made on potted plants of different cultivars of American and Egyptian cotton using the inoculum from *E. heterophylla* and from *F. bona* leaves (Table 2). The test was finally applied in the field on young cotton leaves lightly inoculated at random (to counteract subinfections) on the plant with conidia from *E. heterophylla* and *F. bona*. Every other plant was inoculated along a row of twenty plants but only one row in three was treated, the other two being left as a barrier separating inoculated rows. The plots inoculated with conidia from *E. heterophylla* were separated from those receiving conidia from *F. bona* in such a way as to reduce to the minimum possible dispersal of spores from one plot to the other. The result of this experiment again showed that the conidia from the two hosts were capable of infecting cotton leaves. The conidia from those cotton leaves were found to be infective on cotton as well as on *E. heterophylla* or *F. bona* leaves. At the time of sampling practically none of the uninoculated leaves was infected.

Measurement of conidia. The length and width of conidia from some of the infected host plants were measured. The means of length and width of conidia from each host species represent the data obtained from sampling over 500 conidia from each host throughout the different seasons of the year, and also from plants grown under unnatural conditions (e.g. *F. bona* during August and September in the shade). Furthermore, samples of conidia from the artificially inoculated leaves that became infected (described above) were also measured. Some measurements of conidia from herbarium specimens at the Commonwealth Mycological Institute, Kew, were also made.

Results

Host range. Symptoms appeared 7–12 days after inoculation both in the field and on pot plants. It was found that conidia taken from any one of the hosts, viz. *E. heterophylla*, *F. bona*, *G. barbadense* and *G. hirsutum*, could infect that host and also the other three species (Table 1). Five Egyptian and two American cultivars of cotton were susceptible in varying degrees to infection when inoculated from diseased leaves of *E. heterophylla* or *F. bona* (Table 2). Other cotton cultivars (BAR 4/16, BAR 5/6, SUS 7/2) were also inoculated from the same source; although they appeared to be susceptible, the results were not conclusive. Infection was never established on any other alternative host after inoculation with conidia from *Abutilon figarianum*. Infectivity of the conidia from *Aristolochia bracteata* was tested

Table 1. *Results of cross-inoculation tests, as number of half-leaves (or leaflets) infected of twenty inoculated*

	Conidia of *L. taurica* taken from					
Species inoculated	*E. heterophylla*	*A. figarianum*	*A. bracteata*	*F. bona*	*G. barbadense*	*G. hirsutum*
E. heterophylla	15	0	?4	17	15	13
F. bona	18	0	?2	15	18	10
G. barbadense (Domain Sakel)	14	0	—	18	17	12
G. hirsutum (var BAR 7/8)	12	0	—	14	9	13

— = no test.

Table 2. *Results of cross-inoculation tests as number of half-leaves infected of twenty inoculated*

	L. taurica conidia obtained from			
	E. heterophylla		*F. bona*	
Species inoculated	Cotyledon leaf	First leaf	Cotyledon leaf	First leaf
G. barbadense				
BLR 14/25	14	8	12	16
Domains Sakel	12	10	14	11
XL1	19	14	10	10
X1730	18	8	15	12
Karnak	20	12	16	14
G. hirsutum				
BAR 7/8	15	8	14	10
SUS 16/1	18	8	12	3

on two other host species (*E. heterophylla* and *F. bona*), but results were inconclusive.

Dimensions of conidia. Although conidia may vary in size, the variability was found to be equally great amongst conidia from the same sample as amongst conidia obtained from a different species of host plant. The mean range of length and width of conidia from the different hosts was similar, except for those from *A. figarianum*; the conidia from this species were invariably shorter. This result was obtained from fresh material collected at Shambat, Wad Medani, Shendi (in the Sudan) during various seasons and also from herbarium specimens at the C.M.I., Kew (Table 3).

Table 3. *Frequency distribution of conidial lengths in* Leveillula taurica (*expressed as percentages*)

Range of length (μ)	*E. heterophylla*	*F. bona*	*G. barbadense*	*G. hirsutum*	*A. figarianum*	*C. tinctorius*	*Corchorus* sp.
25–39	—	—	—	—	42	—	—
40–49	12	16	11	12	36	15	8
50–59	53	63	55	48	21	45	56
60–79	34	20	31	38	1	37	35
80–95	1	1	3	2	—	3	1

The width at the broadest line of the conidia from all the host species recorded above was 14–20 μ.

Discussion

The ability of *L. taurica* conidia collected from various host species to infect others has been demonstrated. The mildew is unspecialized, in so far as one 'strain' can infect genera as widely divergent as *Gossypium*, *Euphorbia* and *Faba*.

These findings are not in agreement with those of Zwirn (1943) or Kamat & Patel (1948) who consider this parasite as strictly specialized, and that each strain is infective only to one species of host plant. Zwirn (in Palestine) found that conidia from *Capparis spinosa* L. are able to 'infect *Capparis* plants but not *Passiflora* or *Foeniculum*'. He worked with twelve strains from different hosts and 'encountered this marked specialization in every case'. Zwirn found also that the coefficient (length/width) of the conidia from two species of *Teucrium* was nearly equal, and he presumed that 'apparently the forms on the different species of a single genus are identical', but 'there would be a danger of mutual infection between tomato, egg plant and potato'. This last statement appears contradictory to the theme of Zwirn's argument, since it implies that *L. taurica* may be a 'semi-generalized' parasite. Table 3 shows that all the host leaves, with the exception of those of *A. figarianum*, bear conidia showing the same range of size variability (45–90 × 14–20 μ). In contradiction to Zwirn's findings, there was no distinct 'grouping' in length and width (or presumably of coefficient length/width) of the conidia from different hosts, if the conidia from *A. figarianum* are excluded. In this respect my results are in agreement with those of Kamat & Patel (1948) who found 'the conidia of *L. taurica* from all the hosts (11 species) fall within the range of 39–87 μ which is also typical of *Oidiopsis taurica* (= *L. taurica*) on *Cyamopsis* *psoraloides* DC.'. From their cross-inoculation tests, however, Kamat & Patel found that this parasite is strictly specialized to one species of host plant.

It is apparent that *L. taurica* differs from most powdery mildews in the ability of a single strain to infect a selection of very different host genera (cf. Hammarlund, 1945). However, the conidia from *A. figarianum* were invariably non-infective on other host species and it is believed that this is a distinct species.

Further work remains to be done on the nature of this parasite and on its spread under natural and under controlled conditions. However, it can be stated that *L. taurica* differs fundamentally from most other powdery mildews in that (*a*) it is an endoparasite, the association with the host is not limited to the epidermal layer, as the mycelium ramifies in the mesophyll; (*b*) its path of infection is through the stomata and not the cuticle; and (*c*) under Shambat conditions, it appears to be much less specialized than other powdery mildews. With the knowledge that the infectivity of *L. taurica* is not confined even within the limits of a single host genus, the problem of how primary infection is established, when cleistothecia are rare or absent, becomes less difficult.

One of the most extensively distributed weeds of cultivation in the 'riverain' areas of northern Sudan is *E. heterophylla*. During the surveys made during 1953–5 at Khartoum and Kassala and in the Northern Province of the Sudan, this weed was invariably found to be infected with *L. taurica*. It is highly probable, therefore, that *E. heterophylla* acts as a 'carrier' of the disease during the summer and 'Kharif' rainy seasons, passing the infection in the conidial form to the winter-sown susceptible crops, e.g. broad bean.

EFFECT OF HUMIDITY AND WASHING ON POWDERY MILDEW DISTRIBUTION AND INTENSITY

Members of the Erysiphaceae are of world wide distribution upon mono- and dicotyledonous hosts. They are usually considered as xerophilous, developing best in warmer climates. They occur largely in the dry tropical and subtropical climates, as in the Sudan, Egypt and other Middle Eastern countries, and in the Indian subcontinent during the warm winter months. Further north and south from the equator and in higher altitudes they are predominant during the warm spring and summer months. In general, comparatively low relative humidities (R.H.) are associated with warm weather, when these parasites spread most rapidly. The ability of the powdery mildews to spread under dry climatic conditions may be largely due to the capacity of their conidia to germinate at lower humidities than most, if not all, the other parasitic fungi (Brodie, 1945; Cherewick, 1944; Longree, 1939; Yarwood, 1952).

Erysiphe graminis, with a wide distribution in the cool temperate and Mediterranean climates, is said to be favoured by dry warm weather. Last (1955*a*) reports that the mildew on spring-sown cereals at Rothamsted did not increase greatly until after the end of May when the temperature rose, and presumably the humidity decreased. The concentration of *Erysiphe* spores in the air reached its peak in June and July, during dry weather conditions when sampled at 16.00 G.M.T. (Last, 1955*b*). However, Grainger (1947), in Scotland, found that the incidence of *E. graminis* was greater on the lower leaves of oat crops than on the upper leaves and this he associated with the higher humidity within the crop. The severity of mildew attack in the field in the summer seems to owe more to the increase in relative humidity than to the increase in temperature, and Grainger suspected that the disease became severe when atmospheric humidity remained at saturation level for appreciable periods. According to Tapke (1953), however, there is little or no direct relationship between the spread of *E. graminis* on barley and its ecoclimatic conditions, the severity of the disease being greater on old lower leaves even when the plants were widely separated and in free moving air currents. When the pots containing the barley plants were inverted, the 'upper' older leaves were more severely infected, indicating, therefore, that the greater susceptibility of the lower leaves in barley was due to inherent qualities of the leaf and not to the humidity factor.

In diseases where high humidity is the major determining factor, e.g. potato blight, special precautions need to be taken to maintain a steady high humidity in laboratory experiments when infection is desired. For powdery mildews, however, the infection can be established without such precautions. Indeed, where a water drop is present the germ tubes from the conidia (on glass slides) tend to grow upwards (Dickinson, 1949; Corner, 1935, etc.). Dickinson claims that because leaf surfaces are often covered with water in wet seasons (resulting presumably in the upward growth of germ tubes), the intensity of powdery mildew infection is less than in drier seasons. The results obtained from the present work, however, do not support Dickinson's deduction.

In the Sudan, Boughey (1947) investigated the effect of mean annual rainfall upon the distribution of powdery mildews as one of six plant disease 'groups'. He obtained from field collections the ratio of the actual record of disease in some of the Sudan Provinces, to the 'total potential' record for the same province, the 'total potential' being the sum of all the hosts susceptible in that province whether they were actually attacked or not. The ratio, 'percentage provincial occurrence', was found for powdery mildews to be inversely related to the mean annual rainfall for each province.

Experimental technique and results

With Leveillula taurica (*at Shambat*). Plants of *E. heterophylla* L. in pots were used for experiments during May–September of 1954 and 1955. This species has been chosen because it is a highly susceptible and common weed in northern Sudan, and, being a shade-loving plant, it lends itself better than other hosts to handling in 'Mashtals' (bamboo-shaded huts used for plant propagation and especially suitable in the hot climate of northern Sudan). The tests were made once a month, hence a total of ten repeats was done.

Healthy plants of *E. heterophylla* were transplanted, four in each pot, in the Mashtal. After a week, three pots were used for each treatment; ten leaves per treatment were inoculated with *L. taurica* conidia by gently adpressing diseased lesions on the healthy leaf surface. The treatments were as follows: (*a*) leaves washed immediately after inoculation; (*b*) leaves washed 1 hr. after inoculation; (*c*) leaves washed 3 hr. after inoculation; (*d*) leaves washed 6 hr. after inoculation; (*e*) leaves washed 12 hr. after inoculation; (*f*) leaves washed 24 hr. after inoculation; (*g*) leaves unwashed (control). After treatment, the pots were kept in the Mashtal.

A similar set of treatments (*h–n*) was started on the same day, but the pots were placed immediately after treatment under bell jars, the inner walls of which were moistened, in the Mashtal.

In washing inoculated leaves, a watering can fitted with a rose was used to simulate rain, each treated leaf being washed (but not rubbed) separately with about 500 ml. of water. The leaves were then gently shaken to remove droplets which might contain conidia. The inoculated leaves were washed again 24 hr. after they received their first wetting.

The extent of infection, expressed as 'mildew rating' was assessed by the percentage of leaf area covered, which was estimated when the disease first appeared on each leaf. Mean disease ratings (each based on assessments of ten inoculated leaves) are given in Table 4.

With Erysiphe graminis (*at Rothamsted*). Four tests were made on first and second leaves of young barley plants in pots during June–August 1956. The plants were dusted lightly with fresh conidia by gently tapping a camel-hair brush loaded with the spores 3 in. above the upper surface of the healthy leaf. This method was adopted to ensure that the conidia settled on the leaf surface in a natural manner. The washing was done immediately after inoculation in one treatment and 1, 3, 4, 6, 12, 20 or 30 hr., respectively, after inoculation in the other treatments. One set of treatments was kept freely exposed in a glasshouse and the other at 100% R.H. under bell jars in the same glasshouse. The number of pustules present on the inoculated upper surface was counted on the fifth day and was expressed as the number per 1 cm. length of leaf.

The washing of barley leaves after inoculation was done in two stages. Each leaf was sprayed thoroughly several times with a fine jet of water from a drawn-out glass tube fitted into a plastic wash-bottle. The pots were then placed together and more water was poured on the plants through the rose of a watering can; each pot (containing five plants) received about 500 ml. in the second washing. The leaves were then gently shaken. They were again washed 24 hr. later.

The data obtained from the four tests were broadly similar, and the results given below (Fig. 1) represent the means for each treatment of the last test done on 27 August 1956. The percentage mean of infection for each treatment was calculated according to the formula

$$\frac{mT}{mC} \times 100,$$

where mT is the mean of the treatment total of pustules produced per 1 cm. length of leaf, and mC is the mean of the control total of pustules per 1 cm. length, the control being inoculated but unwashed leaves.

The results of both experiments on *L. taurica* and *E. graminis* closely

Table 4. *Mildew ratings of washed* Euphorbia heterophylla *leaves infected by* Leveillula taurica

Time after inoculation when leaves were washed	May 1954	June 1954	July 1954	Aug. 1954	Sept. 1954	May 1955	June 1955	July 1955	Aug. 1955	Sept. 1955	Mean
(*a*) Less than 1 hr.											
(i) Under bell jar	1	0	1	0	0	0	1	0	0	0	0·33
(ii) In Mashtal	0	0	0	0	0	1	1	0	0	0	0·20
(*b*) 1 hr.											
(i) Under bell jar	0	0	0	0	0	0	0	1	1	0	0·20
(ii) In Mashtal	1	0	0	0	0	0	0	1	1	0	0·33
(*c*) 3 hr.											
(i) Under bell jar	1	1	0	1	2	1	1	2	2	2	1·3
(ii) In Mashtal	2	1	0	1	2	2	1	0	2	2	1·3
(*d*) 6 hr.											
(i) Under bell jar	2	2	2	0	2	2	2	1	1	2	1·6
(ii) In Mashtal	2	2	2	0	1	2	1	1	2	2	1·5
(*e*) 12 hr.											
(i) Under bell jar	2	2	2	2	0	1	2	1	2	1	1·5
(ii) In Mashtal	2	3	2	2	2	2	2	2	2	1	2·0
(*f*) 24 hr.											
(i) Under bell jar	2	2	2	2	2	2	3	3	2	1	2·1
(ii) In Mashtal	2	2	2	2	2	3	3	2	2	2	2·2
(*g*) Control											
(i) Under bell jar	2	2	2	1	2	0	0	2	2	2	1·5
(ii) In Mashtal	2	1	2	2	2	2	2	2	2	3	2·0

Mildew ratings of 0, 1, 2, 3, and 4 correspond to <20, 20, 50, 75 and >75 %, respectively, of leaf area infected.

resemble each other, although methods of assessment were different. For *L. taurica*, the infection was established on most inoculated leaves when washed not earlier than 3 hr. after inoculation, and the increase in extent of the disease with longer intervals between inoculation and washing was inappreciable. The mean area of infection on the leaves washed after 3 hr. was about 30 %, as compared with 40–50 % for the unwashed 'control' leaves. On the other hand, the leaves washed immediately or only one hour after inoculation were remarkably free from infection (0–10 %). There was no difference in the results obtained from the inoculated leaves that were kept in moist chambers and from those placed in the open air of the Mashtal. The consistency of results obtained during two contrasting seasons (dry summer against humid 'Kharif'), and in two consecutive years, indicates that the external environment exerts little or no influence on the incidence of the disease under the conditions of the experiment.

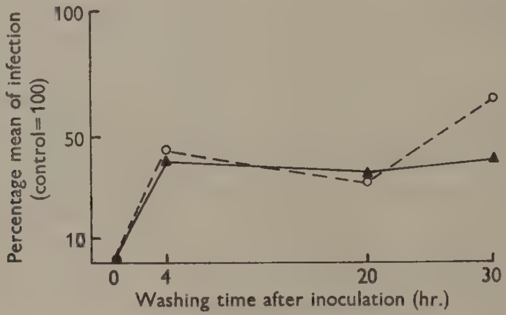

Fig. 1. Effect of washing and high humidity on incidence of *E. graminis* on barley leaves. ▲—▲, Under bell jar (R.H. 100 %); ○- -○, in glasshouse.

Table 5. *Number of pustules of* Erysiphe graminis *per inoculated barley leaf*

	Interval between inoculation and washing (in hr.)															
	0		1		3		4		6		12		20		Not washed	
	UBJ	Free	UBJ	Free	UBJ	Free	UBJ	Free	UBJ	Free	UBJ	Free	UBJ	Free	UBJ	Free
Total	85	110	117	105	460	410	430	395	440	465	740	710	780	855	805	880

(UBJ = under bell jar; Free = in glasshouse.)

There was no difference in the general pattern of results when conidia of *E. graminis* were used. Table 5, representing results from one of the four tests, shows the total number of pustules on the inoculated and washed leaves. Where there is an increase in number of pustules with increase of the interval between inoculation and washing, this is only about twice as much on the unwashed leaves when compared with the 3 hr. treatment but is about four times greater in the 3 hr. when compared with the 1 hr. treatment.

Furthermore, as with *L. taurica*, there is apparently no effect on the incidence of *E. graminis* when the host plant is kept at 100 % R.H. In one test, however, during June 1956, there was a definite lag of 24 hr. in the appearance of pustules on the leaves under the bell jars. However, when they appeared, the number of pustules was closely comparable to that on the leaves in the open air of the glasshouse. It is highly likely that the long sunny spell that coincided with the incubation periods in this particular test caused an appreciable increase of temperature within the bell jars, and that the higher temperature delayed the development of infection.

Discussion

Most of the literature on the effect of humidity on powdery mildews deals primarily with the study of the pathogens as influenced by the atmospheric humidity. Being a variable factor even within the 24 hr. of the day, the conclusions drawn from the atmospheric humidity effect on incidence and spread had to be confirmed by experiments where the humidity was more constantly controlled at one level.

The results of the experiments, both at Shambat and at Rothamsted, show that the two species not only respond in a similar way to high humidity but also thrive well in a saturated closed atmosphere. It is highly probable, therefore, that under natural humid conditions, where the humidity rarely remains constant at 100 % for long periods, conidia of *L. taurica* and *E. graminis* will infect and spread on their host plants if no other factor or factors are limiting. This infection at a constant relative humidity of 100 % may seem at variance with the reduced germination of conidia on water droplets on slides and the growth of the germ tubes upwards (Corner, 1935). However, perhaps on the leaves, water droplets do not persist as they do when placed on slides or, perhaps, there is some stimulus from the leaf inducing the development of appressoria thus leading to infection. The powdery mildews in the Sudan—and in other countries—occur and develop well over a wide range of atmospheric humidities, but they are at the same time well adapted to dry conditions if other factors are favourable.

Boughey's (1947) results give an over-all picture of the extent of the powdery mildew diseases in the Sudan. His results show inverse correlation of their distribution with the mean annual rainfall. He attributed this relationship to several possibilities: (*a*) the washing of conidia by rain from leaf surfaces and from the air; (*b*) the increased occurrence of *Cicinnobolus cesatii* parasitizing the mildews under more humid conditions; and (*c*) the possibility of check on the penetration of infection hyphae as a result of the dilution by rain of the enzyme which dissolves the host cell wall (Woodward, 1927). This last possibility is not generally accepted now. However, not only do the powdery mildews as a group vary in their responses to humidity and show remarkable 'flexibility', but single species and particularly those with a wide host range (e.g. *L. taurica*, *Sphaerotheca fuliginea* and *E. polygoni*) also respond differently to variations in climatic conditions. Furthermore, in an assessment of this nature, within the rain belts of the Sudan or outside, it is the distribution and frequency of the appropriate *host* to a large degree which seems to condition the distribution of the fungus. This may apply to many of the parasitic fungi in general, but it applies much more when the fungus is an obligate parasite, rarely producing a resistant resting stage and largely, if not entirely, disseminated by short lived asexual conidia.

The ability of conidia of *L. taurica* and *E. graminis* to resist the washing effect of rain, well before entry of the infection hyphae, should be emphasized. Most studies on the nature of mildew infection reveal that, although germination may start within the first 6 hr., infection hyphae and haustoria are produced 24 and 48 hr. respectively after inoculation at a temperature of *c.* 20° C. in a saturated atmosphere (Graf-Marin, 1934; Corner, 1935). The ability of conidia to resist washing even before the formation of infection hyphae, as demonstrated by these tests, can be explained only by the assumption that at this stage the conidia and the short germ tubes themselves probably exude a sticky substance which 'glues' them on to the leaf. If this is so with powdery mildews in general and under natural conditions, it may add more evidence of the adaptability of this group to a wide range of environmental conditions.

EFFECT OF HUMIDITY AND WATER ON CONIDIA OF *LEVEILLULA TAURICA* AND OTHER POWDERY MILDEWS

The minimum relative humidity for germination of most fungal spores on glass slides is over 95 %, but conidia of some species of the Erysiphaceae germinate at much lower humidities. In *E. graminis*, Smith & Blair (1950) observed germination over the range 40–100 % R.H., and Cherewick (1944) even claimed that germination occurred at 0 % R.H. On the other hand, Grainger (1947), working in Scotland, found that conidia of the same species showed only 1 % germination at 90 % R.H. on glass slides but failed to germinate in drier air. In *E. polygoni*, as for *E. graminis*, Clayton (1942) found germination at 0 % in apparent confirmation of Yarwood (1936) who claimed, however, that germination was better, sometimes, at 0 % than at 100 % R.H. According to Yarwood (1952), shrinkage of the conidia is associated with germination at low humidity, but Brodie & Neufeld (1942) and Brodie (1945) claim that germination at low humidities is not accompanied by spore shrinkage.

Germination tests on leaves, as opposed to slides, indicate higher germinability (Longree, 1939).

The poor germination of the conidia of powdery mildews on a water surface and the peculiar upward growth of their germ tubes away from such a surface has been reported by several workers (Corner, 1935; Cherewick, 1944; Dickinson, 1949).

The work reported below was undertaken in an attempt to find out how far the water relations of germination of the conidia explain certain features of the occurrence of powdery mildews in the Sudan where infection and spread may occur under exceptionally dry conditions on the one hand, but also under relatively high humidities on the other.

Experimental methods

Germination tests were made on specially prepared ruled slides to facilitate counting. Each slide had etched on it a grid 2 × 3 cm., subdivided into squares of 2 mm. side. The ruled dry slides were thinly coated with conidia and placed horizontally in desiccators over sulphuric acid (Solomon, 1951) or salt solutions (Spencer, 1926) to give appropriate humidities. Another technique was also tested with a view to minimizing the effect of humidity gradients within the containers. In this, a glass slide was suspended by a thread inside a flask containing the humidity-controlling liquid. By agitating from time to time the humidity within the flask was equalized. No discernible differences, however, were observed between the two techniques, but the second was adopted in the final tests. Germination tests at 100 % R.H. and on conidia either floating or submerged were made by placing a drop of water on the middle of a ruled slide powdered with spores. The conidia beyond the edge of the drop were considered as 'dry'. Where the drop was placed, most of the conidia floated and remained unwetted (floating), but some stuck to the glass surface (submerged). The slides were then placed on moist filter-paper in closed Petri dishes, thus maintaining the 'dry' conidia at 100 % R.H. Minute amounts of condensed water often became associated with the 'dry' conidia, but not to the extent that they became submerged or floated. Three slides were used for each treatment, the same diseased leaves being used as a source of conidia for all the humidities tested in one experiment.

The fungal species used, as well as the host species from which conidia were obtained, are given in Table 6. Most tests were done at Shambat (Faculty of Agriculture), but those on *E. graminis* were done at Rothamsted.

Table 6. *Fungal species used in tests, with host plants from which conidia were taken*

Fungal species	Host species
Leveillula taurica (Lev.) Arn.	(1) *Euphorbia heterophylla* L.
	(2) *Abutilon figarianum* Webb.
	(3) *Faba bona* Medic. (= *Vicia faba* L.)
Sphaerotheca fuliginea Schlecht (Poll.)	(1) *Cucurbita pepo* L.
	(2) *Cucumis sativus* L.
Erysiphe graminis DC.	(1) *Hordeum vulgare* L.
E. umbelliferarum de Bary. (= ?*E. polygoni* DC.)	(1) *F. bona* Medic.
	(2) *Daucus carota* L.
E. cichoracearum DC.	(1) *Hibiscus esculentus* L.

On each slide, some squares were selected at random, and their location and the number of conidia within each were recorded, together with the number of spores which had already germinated. This final precaution was necessary because in a spore deposit obtained from mildewed leaves some spores were usually found to have germinated already. However, the percentage of these was normally very low. 300–500 conidia were sampled per treatment. Where no specific time is mentioned, the subsequent recording was done after 24 hr. and again after 48 hr. As has been noted by others (e.g. Corner, 1935), the irregularity in germination, sometimes poor even at optimum R.H., necessitated several repetitions of each test.

The humidities tested ranged from 0 to 100% R.H. Most of the work was done on conidia of *L. taurica* in the Sudan, with the temperature varying according to season between a mean of 22° C. in the colder season to one of 31° C. in the summer (Table 7). At Rothamsted, the conidia from barley (cv. Plumage Archer) were tested at *c.* 15° C.

Three criteria were used in assessing the effect of humidity and water: (1) percentage germination, (2) mean length of germ tube, (3) the state of conidia, whether turgid or shrunken.

Experimental results

With Leveillula taurica. Although some germination occurred at R.H. 0–30%, the percentage was usually very low, the germ tubes were short (1–5 μ) and there was considerable shrinkage of the conidia and their germ tubes. (Fig. 2). Even though these conidia are counted as

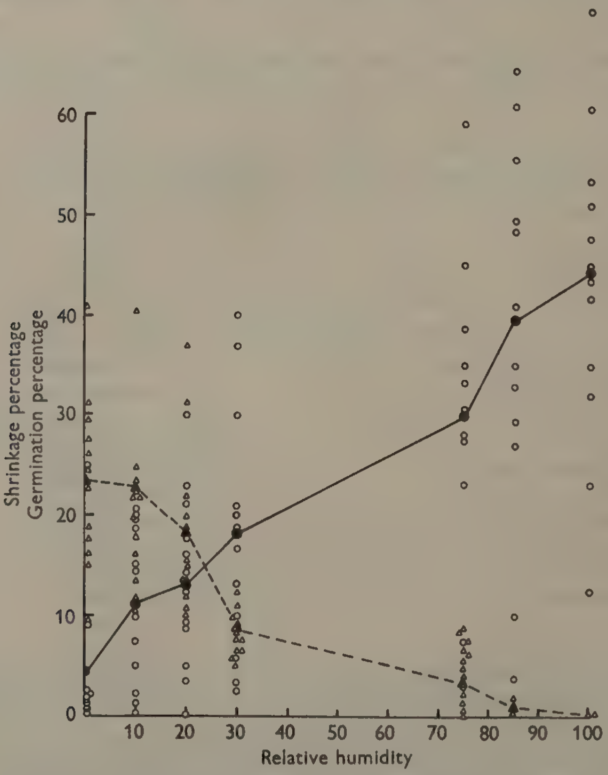

Fig. 2. Germination of *L. taurica* (from *Euphorbia heterophylla*) at different humidities at room temperature (28–33° C.) after 24 hr.

having germinated when the germ tubes are only 1–5 μ long, the percentage germination at the lower humidities is invariably lower than at 75–100 % R.H. (Table 7) in contrast to the findings of Yarwood (1936) with *E. polygoni*.

Table 7. *Effect of humidity on germination of* Leveillula taurica *conidia*

Date	Approx. temp. (° C.)	0%	10%	20%	30%	75%	85%	100%
Conidia from *Euphorbia heterophylla*								
Nov. 1953	25	0	2	8	6	36	15	78
Dec. 1953	23	7	9	0	7	28	16	45
Jan. 1954	22	5	4	23	0	47	39	20
Mar. 1954	24	14	0	10	20	27	44	15
June 1954	31	0	5	10	5	18	68	40
July 1955	29	0	0	3	8	41	24	63
	Mean	4·3	3·3	9	7·6	32·8	34·3	43·5
Conidia from *Abutilon figarianum*								
Dec. 1953	—	0	0	0	0	0	0	0
Feb. 1954	22	0	0	0	15	15	43	38
Apr. 1954	28	6	4	5	1	36	34	20
June 1954	—	9	11	2	5	20	16	61
Aug. 1955	29	8	0	13	28	48	10	40
	Mean	4·6	3	4	9·8	23·8	20·6	31·8
Conidia from *Faba bona*								
Jan. 1954	—	1	2	15	6	32	53	44
Feb. 1954	—	1	4	5	4	42	28	60
Sept. 1955*	28	4	1	3	12	10	46	38
Nov. 1955*	25	2	0	0	0	0	0	0
Jan. 1956	23	0	0	8	0	25	18	35
	Mean	1·6	1·4	6·2	4·4	21·8	29·0	35·4

* Grown in the shade for off-season work.

In many tests, there was little or no germination throughout the whole humidity range, despite the fact that the conidia were collected from fresh leaves. Indeed, it was difficult to foretell from the appearance of the conidia whether they were viable. The brief viability of *L. taurica* conidia (as was demonstrated in unpublished work) appears to be governed primarily by inherent factors rather than by major fluctuations of the external environment. Fresh conidia obtained from one host may yield a high germination percentage, but, when collected on the following day from the same host under similar conditions, germination may be negligible.

The effect of humidity on germ tube length is similar to that on percentage germination. (Figs. 3, 4). In general, the higher the humidity the longer the germ tube and the more turgid is the conidium. Occasionally, however, the spores shrank only slightly after germination, and the germ tubes reached up to 20 μ, at 0 % R.H. The branching of germ tubes, frequently occurring at 75–100 % R.H., was not observed at the lower humidities.

At 100 % R.H., bacteria and other fungi, especially *Alternaria tenuis* Nees and *Curvularia lunata* (Wakker) Boedijn., grew profusely upon the dead conidia of *L. taurica* within 48–72 hr. The invariable occurrence of *A. tenuis* may probably be due to its loose association with the Sudan powdery mildews, but not to an active parasitic habit upon them as with *Cicinnobolus* spp. The hyphae of *A. tenuis* were never observed to penetrate conidia with living germ tubes.

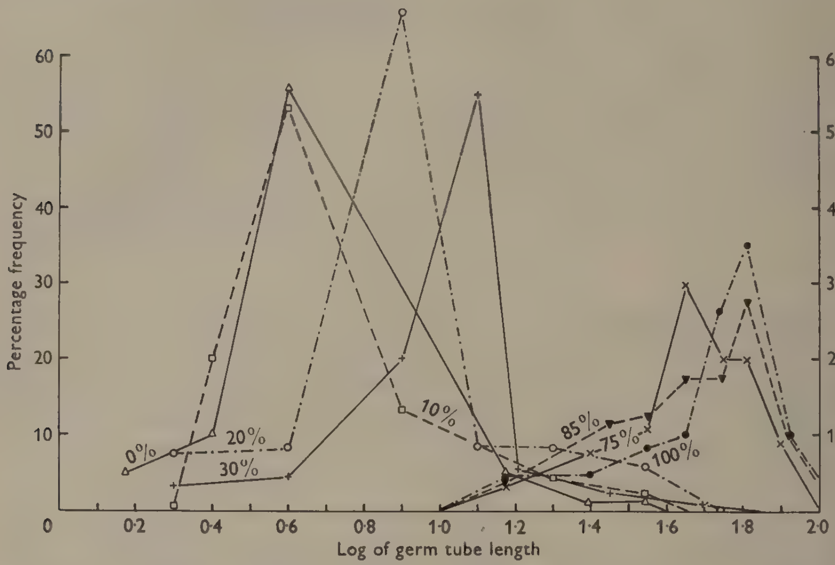

Fig. 3. Effect of humidity on length of germ tube of *L. taurica* conidia after 24 hr. at 28–33° C.

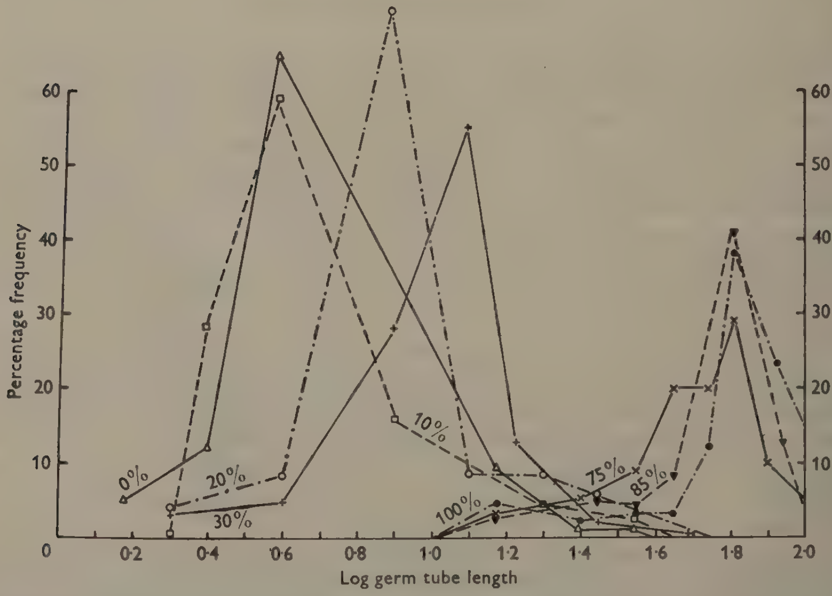

Fig. 4. Effect of humidity on length of germ tube of *L. taurica* conidia after 48 hr. at 28–33° C.

The germ tubes usually arose from near one end but occasionally from the middle of the spore. At 100 % R.H. and on water, a large percentage of the germ tubes grew obliquely upwards, and this effect was seen, but to a lesser extent, at humidities as low as 75 %.

Several tests on germinability of *L. taurica* conidia in or on water were made at Shambat (from *E. heterophylla*) and at Cairo (from *Cynara scolymus* L.). The results (Tables 8, 9) are closely similar; the 'submerged' conidia gave consistently low germination (6 %), whereas germinability of the 'floating' conidia was much higher (22 %) with germ tubes longer than those of the 'submerged' spores; the 'dry' conidia (i.e. at 100 % R.H.) gave 37 % germination.

Table 8. *Effect of liquid water on germination of* Leveillula taurica *conidia at room temperature*

(Results at Shambat after 48 hr.)

	'Floating' conidia		'Submerged' conidia		'Dry' conidia (100 % R.H.)	
Date	Germination	Germ tube length (μ)	Germination	Germ tube length (μ)	Germination	Germ tube length (μ)
Apr. 1955	24	65–120	5	15–40	36	65–180
May 1955	21	50–140	8	18–70	50	45–130
June 1955	18	45–130	3	7–100	44	50–170
July 1955	26	70–140	11	14–60	38	55–160
Aug. 1955	20	50–140	4	12–50	26	20–130
Sept. 1955	16	35–160	10	11–45	53	60–140
Oct. 1955	31	45–130	2	18–60	24	40–160
Nov. 1955	23	40–180	8	20–85	51	30–180
Dec. 1955	25	40–110	8	6–70	28	65–140
Jan. 1956	16	65–130	3	14–60	28	40–130
Feb. 1956	22	30–145	5	8–80	16	30–200
Mar. 1956	27	55–130	9	10–95	50	70–130
Mean	22	120	6	55	37	145

Table 9. *Effect of liquid water on germination of* Leveillula taurica *conidia at* 27·5° C.

(Results at Giza after 48 hr.)

	'Floating' conidia		'Submerged' conidia		'Dry' conidia (100 % R.H.)	
Date	Germination	Germ tube length (μ)	Germination	Germ tube length (μ)	Germination	Germ tube length (μ)
15. v. 56	24	40–180	6	20–100	38	50–160
18. v. 56	28	50–230	14	10–70	59	40–240
Mean	26	115	10	50	49	125

Over 95 % of the germ tubes of the 'floating' conidia grew upwards, reaching a length of over 200 μ in 48 hr. and sometimes branching, but like most germ tubes on glass slides, they were slender and rarely exhibited 'appressorial' swellings. The vertical growth of germ tubes in *E. graminis* and *L. taurica* was found to have no connexion with a response to gravity or light; the mode of germination being similar when the slides were inverted within the moist chamber in the dark. Whatever the conditions on the glass slide, the conidia of *L. taurica* invariably produced a single germ tube.

With Erysiphe graminis. Germinability was tested during the summers of 1955 and 1956 under the following humidities: 10, 22, 40, 60, 80, 95, 98 and 100 %. Although the germ tubes remained short (3–10 μ) after 48 hr. at 95 % R.H., they and their parent conidia were turgid. At 80 % R.H., the percentage germination and the length of germ tubes were only slightly less than at 95 % R.H., but most conidia collapsed. At humidities lower than 80 %, germination was very low or absent (Table 10), and shrinkage and death occurred within 24 hr.

Table 10. *Effect of humidity on germination of* Erysiphe graminis *conidia*

(From Plumage Archer barley at R.E.S.)

Sampling date	Relative humidity (%) 10 G	10 L	10 S	22 G	22 L	22 S	40 G	40 L	40 S	60 G	60 L	60 S
8. vi. 55	0	—	82	0	—	75	2	3	91	6	4	84
15. vi. 55	1	2	94	3	3	86	1	3	84	4	3	78
11. vii. 56	0	—	95	1	3	90	2	3	93	0	—	81
18. vii. 56	0	—	86	0	—	75	3	—	80	5	6	85
9. viii. 56	0	—	93	0	—	83	0	—	70	4	2	75
Mean	0	0	90	1	1	82	2	1	84	4	3	80

Sampling date	Relative humidity (%) 80 G	80 L	80 S	95 G	95 L	95 S	98 G	98 L	98 S	100 G	100 L	100 S
8. vi. 55	22	6	54	26	6	4	16	40	0	71	120	0
15. vi. 55	13	5	60	43	8	11	45	35	0	48	180	0
11. vii. 56	24	5	45	15	8	8	30	65	0	80	130	0
18. vii. 56	25	4	55	30	9	10	25	20	0	55	120	0
9. viii. 56	12	6	50	25	10	4	20	50	0	63	140	0
Mean	19	5	53	28	8	7	27	41	0	63	140	0

G = Percentage germination of conidia; L = Mean length of germ tube (μ); S = percentage shrinkage of conidia.

Of the viable conidia at 95 % R.H., less than 2 % produced two germ tubes and very rarely more than two. Up to 5 or more germ tubes per spore (3–4 average) may be found at 100 % R.H., and the mode of growth of the longest germ tube is either vertical with a long, slender and occasionally branched hypha or horizontal and short with an appressorium.

With Sphaerotheca fuliginea. The conidia showed much more sensitivity to low humidity than those of *L. taurica* (Table 11), no germination below 85 % R.H. being observed. One germ tube was normally produced varying in length between 35 and 65 μ at 100 %, 35 and 42 μ at 95 % and 3 and 10 μ at 85 % R.H.

As with *L. taurica*, many tests gave no germination even at 100 % R.H., indicating short viability. Distortion of the conidial wall occurred below 95 % R.H.

With Erysiphe cichoracearum. The results were similar to those with *S. fuliginea*, with probably a slightly higher tolerance to the lower humidities, but possibly with a shorter viability period. Even at 100 % R.H.

only one germ tube was produced; the length after 48 hr. varied from 3 to 85 μ (av. 32 μ), but at 85 % it was significantly less (3–8 μ).

With Erysiphe umbelliferarum. Although several tests were made during two seasons, the germination was invariably very poor (Table 11). At 100 % R.H. the percentage germination was 5, and at 85 % R.H. 3. Under drier conditions no germination was observed.

Table 11. *Effect of humidity on percentage germination of conidia in various powdery mildew fungi*

	S. fuliginea				*E. cichoracearum*				*E. umbelliferarum*			
	Relative humidity (%)				Relative humidity (%)				Relative humidity (%)			
Date	15	85	95	100	30	75	85	100	75	85	95	100
4. v. 54	—	—	—	—	0	0	8	11	—	—	—	—
30. xi. 54	0	5	10	16	—	—	—	—	—	—	—	—
2. i. 55	0	2	28	38	—	—	—	—	—	—	—	—
20. ii. 55	—	—	—	—	—	—	—	—	0	2	3	5
25. ii. 55	—	—	—	—	—	—	—	—	0	2	2	1
[illegible]. iii. 55	—	—	—	—	—	—	—	—	0	4	2	4
6. iv. 55	0	7	32	32	—	—	—	—	—	—	—	—
6. vii. 55	0	4	5	24	3	4	4	13	—	—	—	—
14. xii. 55	0	0	12	36	1	8	30	33	—	—	—	—
8. iii. 56	—	—	—	—	—	—	—	—	0	8	8	10
10. iii. 56	—	—	—	—	0	0	15	18	0	0	2	6
12. iv. 56	—	—	—	—	1	3	9	6	—	—	—	—
Mean	0	4	17	29	1	3	13	16	0	3	3	5

Discussion

Corner (1935) found that 1–3 hr. in water killed the conidia of *Erysiphe graminis*, *Podosphaera leucotricha*, *Sphaerotheca pannosa*, *Erysiphe cichoracearum* and *Oidium* spp., but floating conidia germinated readily after 24 hr., and produced upright germ tubes 100–200 μ in length. No mention is made, however, of the fewer appressoria produced and the reduction in germination percentage when conidia of *E. graminis* are floating, as compared with germination at 100 % R.H. when the spores are not associated with water droplets of any considerable size. On the slides maintained at 100 % R.H., although some water condenses around the spores, it is not sufficient to submerge them or to cause them to float.

Corner suggests that the growth of germ tubes away from free moisture under saturated conditions is due to their negative chemotropism to carbon dioxide. He believes that this quality is also a function of the hyphae of powdery mildews 'wherefore they cannot normally enter stomata or become endoparasites, but for the most part are compelled to lead an extoparasitic existence'. The germ tubes of *L. taurica* (an endoparasite) also grew away from the water surface. Dickinson (1949), however, found that germ tubes of other endophytic obligate parasites as well, e.g. *Puccinia triticina*, grew away from a water surface, and that the direction of growth is conditioned by the nature of the 'membrane'.

Carbon dioxide may affect the actual germination rather than the direction of growth of the germ tube. It was observed that the percentage germination is usually lower where the conidia are crowded than where they are relatively far apart; the larger mass of conidia per unit area produces more carbon dioxide in respiration and thus possibly increases the inhibition of germination. According to Domsch (1954), however, germination is poor, when the conidia are crowded, owing to the exudation by the conidia themselves of a toxic agent other than carbon dioxide. Further work, therefore, is required on this aspect of mildew physiology. Furthermore, what causes the production of long, slender and often upright germ tubes with no appressoria by some conidia of *E. graminis*, and of short germ tubes with appressorial swellings by others under the same conditions, is not fully understood. Again where germination of *L. taurica* conidia was tested on leaf as well as on slide surfaces (unpublished work), not only was the percentage higher on the leaf but the germ tubes themselves were different; they grew along the surface, branched freely and produced 'anchoring hyphae'.

The conidia of some powdery mildews are able to germinate on water films, and although appressoria are rare on large water drops, they are abundantly produced at 100 % R.H. where a water film condenses on the slide and, hence on the conidia themselves. The ability, therefore, of a large number of germ tubes to produce appressoria on *shallow* water films is an indication that infection could probably take place had the surface been a host leaf and not a glass slide. Indeed, in a different experiment infection by *E. graminis* was established on barley (and by *L. taurica* on *E. heterophylla*) when the inoculated and moistened leaves were placed immediately at 100 % R.H. Under natural conditions, however, the humidity may remain high or at saturation point for long periods, but not usually throughout 24 hr. Nevertheless, even when the humidity is maintained at saturation point, it is apparent that infection and establishment of the disease can and does take place.

At Shambat, tests on the effect of water on germination of *L. taurica* conidia were done in all the months of the year. The ability of conidia to germinate during the hot summer (May–July) or 'Kharif' rainy season (July–September) may partly account, therefore, for the prevalence of the disease on weed hosts, e.g. *E. heterophylla*, throughout the whole year.

The effect of humidity on conidial germination of powdery mildews in the Sudan is similar to that reported from other countries. The findings here are in agreement with those of Yarwood (1952) who found that conidia of *E. polygoni* have a much higher water content than conidia of fungi other than powdery mildews. The shrinkage associated with germination at low humidities could hardly be explained otherwise.

Conidia of *S. fuliginea*, *E. cichoracearum* and of *E. graminis* (from barley at R.E.S.) show higher sensitivity to dryness. Hashioka (1937) reported that no germination of *S. fuliginea* conidia was observed at 99 % R.H. or under. However, conidia of *E. umbelliferarum* (which are apparently very short-lived) gave poor germination even at saturation. Nevertheless, since there was appreciable germination at the higher humidities, at least for *E. cichoracearum* and *S. fuliginea*, and none at the lower humidities, it is evident that the conidia of these fungi are much more sensitive than those of *L. taurica*.

The germination tests at different humidities on glass slides might indicate, to a limited extent, the behaviour of conidia under natural conditions on leaf surfaces. When compared with conidial germination of other fungi on glass slides at different humidities, the conidia of powdery mildews show marked xerophilous characteristics. However, even on glass slides, the powdery mildew conidia germinate best between 95 and 100 % R.H. (Longree, 1939; Grainger, 1947). Only at these humidities and to a lesser extent on water, is their germination (at least for *E. graminis*, from barley at Rothamsted) comparable to that on leaf surfaces under natural conditions. At 100 % R.H., these conidia abundantly produce appressoria within 48 hr., whereas few appressoria are formed below 98 %. So although the conidia of powdery mildews can germinate at much lower humidities on glass slides than those of other fungi, yet it is unlikely that these conidia are capable of causing infection within the wide range of humidity permitting actual germination.

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THE PHYSIOLOGY OF SPORULATION IN *PENICILLIUM GRISEOFULVUM* DIERCKX

BY A. G. MORTON, D. J. F. ENGLAND
AND DAPHNE A. TOWLER

Imperial Chemical Industries Limited, Akers Research Laboratories, Welwyn, Herts

The conditions for sporulation of *Penicillium griseofulvum* were investigated. Sporulation does not occur in submerged culture in a glucose-nitrate medium but can be induced (in appropriate conditions) by the following methods: transfer of mycelium to aerial conditions, increasing the concentration of sugar in the medium, addition of calcium, copper, or crude glucose, partial desiccation of the mycelium, transfer of mycelium to nitrogen-free medium.

P. griseofulvum may be induced to sporulate at a very early stage of germination but not before it has assimilated some external nitrogen. Induction of sporulation can be reversed by adding assimilable nitrogen sufficiently soon after induction.

Sporulation of four other species of *Penicillium* and two species of *Aspergillus* was induced by some, but not all, of the conditions found effective for *Penicillium griseofulvum*.

INTRODUCTION

Many fungi reproduce mainly or exclusively by the formation of asexual conidia. In spite of the biological and economic importance of this process, the external and internal factors which control it are little known. Sporulation seems often to be favoured by those conditions, such as falling or low food supply, which are adverse to mycelial growth, but this is not always the case. Various apparently unrelated factors have been recorded as inducing or stimulating sporulation in different species (Table 1). In a given species the occurrence of sporulation is likely to depend on a complex of factors which together provoke the requisite metabolic processes. A general discussion of the problem of development in fungi has recently been given by Hawker (1956).

The aim of this work was to determine some of the external factors which affect the transition from mycelial growth to sporulation. Attention has been directed to the conditions which induce (or prevent) sporulation, rather than to conditions affecting the number of conidia finally produced. Most of the experiments concern a single strain of *Penicillium griseofulvum*, but some other species were used for comparison. This study is intended as the basis for an investigation of the internal metabolic changes which accompany the transition from vegetative growth to sporulation.

Table 1. *Sporulation in fungi*

Species	Factors inducing or stimulating sporulation	Reference
Melanconium fuligeneum	Concentrated inoculum	Timnick, Barnett & Lilly, 1952
Guignardia bidwellii	Low nutrients	Barnett, Timnick & Lilly, 1950
Septoria nodorum	Low nutrient, 20° C., continuous light	Richards, G. S., 1950
Aspergillus, *Penicillium* spp.	Supra-optimal temperatures for vegetative growth	Muller, 1956
Trichoderma viride	Ca, Fe, Cu	Brian & Hemming, 1950
Penicillium notatum	Ca (in submerged culture)	Foster, McDaniel, Woodruff & Stokes, 1945
P. notatum	Ca, organic nitrogen source, Fe (500–100 μg./l.) (submerged culture)	Gilbert & Hickey, 1946
Trichophyton persicolor	Trace elements	Stockdale, 1953
Aspergillus niger	Trace elements	Steinberg, 1919, 1939
Colletotrichum lindemuthianum	Peptone, vitamins	Mathur, 1949
Pilobolus sp.	Light, thiazole	Page, 1952
Choanephora cucurbitarum	Alternation of light and dark, or continuous low light	Barnett & Lilly, 1950
Trichoderma lignorum	Brief light (no sporulation in complete darkness)	Miller, Webb & Reid, 1953
Alternaria spp.	Ozone	Richards, M. C., 1949
Penicillium spp.	Strain specificity (submerged culture)	Grosser, Kundtner-Schwarzkopf & Bernhauer, 1950
Aspergillus oryzae	Subaerial conditions, relative humidity 90–95 %	Feniksova & Segal, 1953

MATERIALS AND METHODS

The following fungi were used in the investigation:

ARL culture collection no.	
375	*Penicillium griseofulvum* Dierckx
585	*P. frequentans* Westl.
739	*P. thomii* Maire
594	*P. melinii* Thom
476	*Aspergillus nidulans* (Eidam) Wint.
32	*A. niger* van Tiegh.

Subcultures were made on Czapek-Dox agar in flat bottles in order to provide a supply of spores. The spores were suspended in sterile water and aliquots of the suspension were used for inoculation. The inoculum was not closely standardized but was such as to give about 10^6 spores per ml. medium after inoculation.

The culture medium in most experiments had the following composition: 50 g. glucose (A.R.); 1·0 g. KH_2PO_4; 0·5 g. $MgSO_4.7H_2O$; 2·3 g. KNO_3; trace elements (200 μg. Fe, 38 μg. Cu, 225 μg. Zn, 24 μg. Mn, and 40 μg. Mo); water to 1 l. The pH was adjusted initially to 6·5. This will be designated as 5 % glucose-nitrate medium; variations in composition in particular experiments will be mentioned later. In most experiments the fungus was grown in submerged culture in flasks on a reciprocating shaker; each flask contained 200 ml. culture medium and was plugged with cotton-wool. The temperature of incubation was 25° C. The course of growth and sporulation was followed microscopically at intervals. Measurements of increase in dry weight of the fungus, rate of assimilation of nitrate and pH of the medium were made as required, according to methods previously described (Morton & MacMillan, 1954; Morton & Broadbent, 1955).

The induction of sporulation was considered positive when it occurred in a significant proportion of the mycelium present. In the heavily seeded cultures employed, the mycelium (thallus) derived from individual spores can generally be distinguished, and it is possible by direct counting to estimate roughly the proportion of individual mycelia forming conidiophores. Most frequently 70–90 % of the mycelia form conidiophores after induction, although figures as low as 20 % may be observed, and were considered significant when induced regularly.

It may be noted that when *P. griseofulvum* produces conidia in submerged culture, the conidia do not germinate in the culture medium in which they are produced, although they are immediately germinable in a fresh medium. This property makes *P. griseofulvum* convenient material for experiments on sporulation.

EXPERIMENTS WITH *P. GRISEOFULVUM* IN SUBMERGED CULTURE

Spore germination

The recently harvested spores of *P. griseofulvum* show practically 100 % germination within a few hours of inoculation into 5 % glucose-nitrate medium kept aerated by shaking. This level of viability is maintained by air-dry spores, kept at room temperature, for at least two months after harvesting. Spores older than this were not used for inoculation. For germination the simultaneous presence of glucose, KNO_3, PO_4^{3-}, and SO_4^{2-} was shown to be essential; Mg^{2+} and the trace elements were not essential at this stage.

Growth in glucose-nitrate

In the culture conditions employed, rapid mycelial growth and nitrate uptake began 15–20 hr. after inoculation and continued rapidly until all the available nitrogen had been assimilated. Nitrate had usually disappeared from the medium 40–44 hr. after inoculation. Thereafter the total mycelium continued to increase slowly in dry weight for a time, at the expense of the glucose still present in the medium.

Observations were carried on for 5–6 days and showed that the mycelium generally remained completely vegetative; only rarely a few odd conidiophores appeared, not earlier than 4–5 days after inoculation. Thus *P. griseofulvum* does not normally sporulate in submerged culture in 5 % glucose-nitrate and this system is therefore a convenient one for investigating factors inducing sporulation.

Effect of 'crude' glucose

The standard glucose-nitrate medium was made up with 5 % pure glucose (A.R.), and no sporulation took place in submerged culture in this medium. When, however, pure glucose was replaced by a slightly brownish commercial glucose (which we shall designate 'crude' glucose), there was a very marked change in behaviour of the fungus. The rate of growth and nitrate uptake was the same as with pure glucose, but the mycelium began to develop conidiophores as early as 20 hr. after inoculation and was sporing freely, long before all the nitrate had been taken up. Sporulation also occurs in a glucose-nitrate medium containing 4·5 % pure glucose and 0·5 % crude glucose. These facts suggested that crude glucose contains a 'sporing factor', not present in purified glucose. The nature of this factor forms the subject of a separate investigation, and it will only be noted here that crude glucose can in fact be freed from this factor by treatment with a suitable ion-exchange resin; the glucose does not then induce sporulation. The factor, after elution from the resin, will induce *P. griseofulvum* to spore in pure glucose-nitrate medium to which it is added. The factor is not identifiable with any of the common vitamins or with calcium (see below).

Effect of concentration of glucose

When the concentration of (pure) glucose in the medium was varied, the other components remaining unchanged, sporulation was found to take place abundantly if the level of glucose was raised from 5 to 10 % or 20 % (equally effective). Sporulation did not occur at lower concentrations of glucose (5, 1, 0·5 %). This inducing effect of high concentrations of pure glucose might indicate that it also contains the 'sporing factor', but in smaller amount than in crude glucose. However, pure glucose, after treatment with ion-exchange resin to remove the factor, still caused sporulation at concentrations of 10–20 %, whilst no sporing factor could be eluted from the resin. It appears therefore that a high concentration of glucose can in itself cause sporulation of *P. griseofulvum.*

Varying concentrations of crude glucose were also tested in the medium, and it was found that sporulation did not occur when the glucose concentration was reduced to 1 % or under. Since this concentration of crude glucose is capable of inducing sporulation (by virtue of its sporing factor) when the total concentration of glucose (pure + crude) is 5 %, it would appear that a low concentration of glucose in relation to nitrogen supply prevents sporulation, irrespective of the presence of sporing factor. In this connexion it is significant that the fungus is unable to assimilate all the nitrate when less than 2 % glucose is present in the medium.

The relation between the level of carbohydrate and nitrogen is the deciding factor, as was shown by experiments with a crude-glucose nitrate medium of the standard composition but diluted to 1/5. The actual concentration of crude glucose was thus 1 %, but the nitrate concentration was reduced in proportion, and in this medium sporing occurred abundantly. When the nitrate only was raised to its normal level, sporing was completely suppressed; in these conditions the fungus could not assimilate all the nitrate. The failure to sporulate in these conditions is doubtless due to exhaustion of carbohydrate at the stage when sporulation could occur.

In further experiments in 5 % pure glucose-nitrate it was found that sporulation could be induced by adding a higher concentration of glucose at various stages of growth, not necessarily initially. Whereas control cultures in 5 % glucose-nitrate showed no signs of spore formation in 3 or 4 days, the addition of a further 5 % glucose at 20–24 hr. or at 40–44 hr. after inoculation caused general sporulation within 24 hr. of the glucose addition. The induction of sporulation in 40 hr. mycelium is noteworthy, for at this stage all the available nitrogen in the medium has been used. The addition of more glucose thus causes an internal re-mobilization of nitrogen in the mycelium, leading to conidiophore- and spore-formation.

The induction of sporulation is more rapid and complete when 5 % additional glucose is added at a later stage to the culture than when it is present initially. It seems unlikely that induction of sporulation by additional glucose is a nutritional effect, for analysis showed that even in 40–44 hr. cultures there was still abundant glucose present (usually 2–3 %) when 5 % more glucose was added. More probably the primary effect of glucose addition is a physical change in the mycelium, associated with a change in the osmotic water-relations of the protoplasm. This suggestion is strengthened by the fact that sporulation in glucose-nitrate was also fully induced by the addition of 5 % sorbose (at 24 and 40–44 hr.), although sorbose is metabolized with extreme slowness by *P. griseofulvum*, if at all.

Effect of calcium

Several workers have observed that calcium stimulates sporulation in fungi (Table 1). The addition of 0·1–1 % calcium chloride was found to induce sporulation of *P. griseofulvum* in glucose-nitrate. Induction was effective whether the calcium was added to the medium initially or at 40–44 hr., when the mycelium was well grown and had used up all available nitrogen. The effect was shown to depend on the calcium and not on the chloride ion. The effect of calcium could not be produced by increasing the magnesium content of the medium.

Effect of copper

The glucose-nitrate medium used in these experiments contains 6×10^{-8} M copper. It was found that sporulation could be induced by the addition, to 40–44 hr. mycelium, of 5×10^{-5}M copper. At this stage of growth $1–5 \times 10^{-5}$M copper (added as $CuSO_4.5H_2O$) regularly induces sporulation, although the proportion of mycelia sporing tends to be rather lower (20–50 %) than for the methods hitherto described. The range of effective concentration is narrow, as may be seen from Table 2. With younger (24 hr.) mycelium, 5×10^{-5}M copper does not induce sporulation but is found to inhibit growth. Sporulation has not so far been induced in 24 hr. mycelium by the addition of copper, in the few experiments tried.

Table 2. *Effect of added copper on sporulation of* Penicillium griseofulvum

Age of mycelium (hr.) when copper was added	Molarity of added copper	Sporulation
24	1×10^{-6}	−
	4×10^{-6}	−
	1×10^{-5}	−, growth inhibited
	5×10^{-5}	−, growth inhibited
40	1×10^{-6}	−
	4×10^{-6}	−
	1×10^{-5}	+
	5×10^{-5}	+
	5×10^{-4}	−

Effect of transfer to nitrogen-free medium

Sporulation was induced by transferring mycelium growing vegetatively in glucose-nitrate to a medium containing glucose (5 %), KH_2PO_4, $MgSO_4$ and trace elements, in the usual amounts, but lacking KNO_3. The mycelium was filtered off from the original medium, washed with nitrogen-free medium, re-suspended in nitrogen-free medium and replaced on the shaker. Within 24 hr. the transferred mycelium was sporing freely, whilst mycelium left in glucose-nitrate remained vegetative as usual. The proportion of mycelium sporing was usually 20–50 %. During the period in nitrogen-free medium the mycelium increased in dry weight by 50–60 %, showing that carbohydrate is assimilated in these conditions.

The transfer to nitrogen-free medium was most effective in causing sporulation when the mycelium was 24 hr. old or younger. At this stage of growth the available nitrate in the medium is still high, and the transfer to medium lacking nitrogen seems to be the decisive factor in causing the mycelium to spore. Mere transference of mycelium to fresh glucose-nitrate did not induce sporulation. Transfer was effective in causing sporulation even when the glucose concentration of the nitrogen-free medium was reduced to 1 %, i.e. considerably lower than the concentration in the medium in which the fungus was growing before transfer. The inducing effect is thus distinct from that caused by an increase in glucose concentration.

Glucose concentration, nevertheless, showed its effect in such transfer experiments. With 24 hr. mycelium, the induction of sporulation was usually somewhat greater when the transfer was made to nitrogen-free medium with 5 % glucose than to that with 1 % glucose. Mycelium which was 40–44 hr. old only responded markedly to transfer when the nitrogen-free medium contained 10 % glucose. In this case the response must be due to the raised glucose concentration, not to lack of nitrogen, since the fungus had exhausted all available nitrogen in the original medium at the time of transfer.

Effect of brief period of desiccation

Mycelium growing in glucose-nitrate medium was filtered off when about 40 hr. old. It was sucked free of medium as far as possible on a Buchner filter funnel, but was not washed. The pad of mycelium was removed from the filter, weighed, and then kept in a desiccator for $\frac{1}{2}$–1 hr. on a sterile Petri dish at room temperature until between 5 and 10% of the original fresh weight had been lost by drying. The mycelium was then resuspended in the glucose-nitrate medium from which it had been filtered off and the flask was returned to the shaker and incubated further. Within 24 hr., 90% of the mycelium was bearing conidiophores and spores. Control mycelium, which had been filtered off at the same time and returned to the medium without any desiccation, remained vegetative. Mycelium filtered off and kept 2 hr. in air, in conditions that prevented desiccation, also remained vegetative after being returned to its original culture medium. It appears therefore that the stimulus to sporulation lies in some process connected with the drying of the mycelium, rather than with an increased access of oxygen, although more critical investigation of this question is required. The effect of desiccation is evidently not reversed by the return to submerged conditions.

SUBAERIAL CULTURE OF *P. GRISEOFULVUM*

In all the experiments so far described the fungus was grown, and sporulation was induced, in submerged culture. Its behaviour when grown in surface culture on a liquid or solidified medium was also examined. It soon became clear that in these conditions the dominant factor inducing sporulation is the emergence of certain hyphae from the medium into the air. Conidiophores were formed in these conditions irrespective of the presence of pure or 'crude' glucose, or of calcium, or of the concentration of glucose or of nitrate. These latter factors, which certainly alter the amount of growth of mycelium, probably affect the degree of sporulation to some extent, but these effects have not been investigated in detail, since they are obviously of secondary importance in relation to the overriding inductive effect of subaerial or aerial conditions.

In this connexion the results of transferring mycelium from submerged to subaerial conditions are of interest. From a submerged culture in glucose-nitrate, 24 hr. after inoculation, 1–3 ml. volumes of medium with mycelium in suspension were removed and treated in each of three ways: (1) spread in a thin layer to cover the bottom of a sterile Petri dish; (2) spread over the surface of a layer of water agar in a Petri dish; (3) spread over the surface of a porous (porcelain) plate. The cultures were covered to prevent drying out and were incubated at 25° C. Each culture showed abundant sporulation on numerous aerial conidiophores within 24 hr. Submerged mycelium transferred to subaerial conditions at 44 hr. behaved in the same way. In this case the culture medium contained no nitrate, although 1–2% glucose was still present. Mycelium which continued in submerged culture remained vegetative.

In other experiments mycelium from submerged cultures in glucose-nitrate medium was filtered off, washed with nitrogen-free medium, and then resuspended in nitrogen-free medium. Part of the suspension was returned to the shaker and part was placed in subaerial conditions by spreading in a thin layer on water agar. With 24 hr. mycelium, transfer to nitrogen-free medium induced sporulation in submerged culture, as was previously observed, but the proportion of sporing mycelium was only 10–20 %, whereas in subaerial conditions it was practically 100 %. With 40 hr. mycelium, transfer to nitrogen-free medium failed to induce any sporulation in submerged cultures, whereas sporulation was again practically 100 % in subaerial cultures. These experiments show very clearly the preponderant effect of subaerial conditions in the induction of sporulation.

GENERAL CONDITIONS FOR SPORULATION OF *P. GRISEOFULVUM*

Ripeness to spore

Experiments have thus revealed a number of factors inducing formation of conidiophores by vegetative mycelium. The question may be asked, whether mycelium has to reach a certain stage of development before it becomes capable of responding to the inductive stimulus. Spores of *P. griseofulvum* were suspended in glucose-nitrate medium on the shaker at 25° C. Samples of spores were removed by filtration immediately, and at 6, 12, 18 and 24 hr. after inoculation. The spores were thoroughly washed on the filter with 10 % glucose in phosphate buffer (pH 6·9) and were then incubated in this solution on the shaker and examined microscopically at intervals up to 3 days after transfer. Transfer of 24 hr. mycelium to 10 % glucose in phosphate buffer is known to induce sporulation. It was found that spores transferred after 2 min. and after 6 hr. incubation in glucose-nitrate produced short (usually unbranched) hyphae in 10 % glucose-buffer, but did not form conidiophores or spores. Spores transferred after 12 hr. in glucose-nitrate behaved very similarly, but branching of the hyphae occurred occasionally and about 5 % of the mycelia bore terminally normal conidiophores, which produced chains of 2–5 apparently normal spores. When transferred after 18 or 24 hr. in glucose-nitrate, a large proportion of the mycelia formed conidiophores and spores in 10 % glucose-buffer.

It appears, therefore, that there is a very brief period in the early growth of the fungus from the spore when it is not capable of responding to the sporing stimulus. This period is about 12 hr. in the cultural conditions employed. Morphologically it is marked only by some swelling of the germinating spore and sometimes by an indication of the germtube. Any uptake of nitrate, glucose, etc., during this time is too small to be measured by the usual methods; the following experiment provides evidence, however, that the assimilation of certain nutrients is an essential condition for passing this stage of development. Spores were suspended in a series of 'glucose-nitrate media', from each of which in turn a main constituent of the basic medium was omitted. After incubation for 16 hr. at 25° C. on the shaker, the spores from each medium were filtered off, washed with 10 % glucose-buffer, and then shaken in it for a further 48 hr. and examined for conidiophore and spore formation. Sporulation was observed after incubation in complete medium, and in media lacking (1) sulphur (SO_4^{2-}), (2) potassium (replaced by Na^+), (3) magnesium, (4) trace elements. There was no sporulation after transfer from media lacking (1) glucose, (2) nitrate, (3) phosphate, or (4) all nutrients. Thus the development of the fungus to the stage when it becomes capable of sporulation requires an exogenous supply of carbohydrate, nitrogen and phosphorus, and this presumably implies a distinct, although very small, amount of growth.

Nutrients and sporulation

The process of sporulation itself may take place in complete absence of any external assimilable nitrogen, as is clear from many experiments already described (induction in various ways of sporulation in 44 hr. mycelium, transfer of mycelium to nitrogen-free medium, subaerial conditions, etc.). It is essentially a process in which internal nitrogen is remobilized, and the requirements for other nutrients at this stage are of some interest. The transfer of 24 hr. mycelium grown in glucose-nitrate into nitrogen-free medium provides a system for investigating this question, for the various constituents of the medium may be omitted in turn and the effect on sporulation determined. It was found that only glucose and phosphate were essential for sporulation, the other nutrient factors being presumably present in sufficient supply in the mycelium grown in these conditions. Sporulation sometimes occurred in glucose only, without phosphate, but never in phosphate alone. As might be expected, oxygen is required for sporulation, which is completely stopped if air in the culture flask is replaced by nitrogen.

Reversal of induction

Although sporulation may sometimes take place in the presence of a high level of assimilable nitrogen (in crude glucose medium, for example), it is generally associated with the absence of nitrogen (the stage of nitrogen exhaustion). Experiments were therefore undertaken to see whether the addition of nitrogen would prevent the induction of sporing. Using 44 hr. mycelium (all nitrate gone from the medium), sporulation was induced by adding 5 % sorbose, or 5 % glucose, or 1 % $CaCl_2$. Using 24 hr. mycelium, sporulation was induced by transferring the mycelium to nitrogen-free medium. When 0·1 % KNO_3 was added 2, 5 or 7 hr. after the beginning of induction, it completely suppressed sporulation, whatever method of induction had been used, whilst all induced cultures without added nitrate spored vigorously. When nitrate was added 8 or 16 hr. after the beginning of induction, however, sporulation took place as vigorously as in the cultures without added nitrate. The addition of assimilable nitrogen thus inhibits or reverses the induction of sporulation if added sufficiently early, but when the process of induction has reached a certain stage (attained in about 7–8 hr. in the given conditions), it can no longer be inhibited by nitrate. There was no morphological indication of sporulation 8 hr. after the beginning of induction. At 16 hr. after induction, a very few recognizable conidiophores could be observed (in cultures without nitrate), whilst at 24 hr. there were numerous conidiophores bearing conidia.

Ammonia-nitrogen (as ammonium tartrate) prevented sporulation in the same way as nitrate, when added to induced cultures. Both forms of nitrogen were taken up by the mycelium in these conditions, and suppression of sporulation is probably connected with this assimilation of external nitrogen.

EXPERIMENTS WITH OTHER SPECIES

The induction of sporulation was examined in several other fungi in the same series of experimental conditions as were used for *P. griseofulvum*. The results are summarized in Table 3, in which those for *P. griseofulvum* are also included for comparison.

Table 3. *Sporulation of fungi in submerged culture*

	Treatment						
	Basic medium	Crude glucose in basic medium	Basic medium + 1 % $CaCl_2$ during growth	Basic medium + (extra) 5 % glucose during growth	Partial drying of mycelium and return to growth medium	Transfer of mycelium from basic to nitrogen-free medium	Transfer of mycelium to sub-aerial condition
Penicillium griseofulvum	−	+	+	+	+	+	++
P. raistrickii	+	++	++	+	−	+	++
P. frequentans	−	−	(+, 30 hr.) (−, 48 hr.)	+	*	+	++
P. thomii	−	+	++	−	−	−	++
P. melinii	+	++	*	*	*	*	++
Aspergillus nidulans	+	+	−	+	+	−	++
A. niger	−	−	−	−	−	−	++

* = No test.

No sporulation = − ; sporulation = + or ++ ; basic medium = 5 % (A.R.) glucose-nitrate.

Except in their response to subaerial conditions, which in every case induces vigorous sporulation, the species vary in behaviour. Three species produce spores quite freely in the basic medium. Crude glucose induces sporulation in two species, and obviously increases sporulation in two other species, in which, however, it is not essential for induction. Calcium induces sporulation in three species (although in *P. frequentans* its efficacy depends on age of the mycelium), and increases sporulation in one species. *P. frequentans* behaves like *P. griseofulvum* in showing an inductive response to increased glucose concentration and to a nitrogen-free medium, whilst *P. raistrickii* sporulates in a nitrogen-free medium. It is noteworthy that the Aspergilli differ from the Penicillia in relation to calcium. Whilst calcium either induces or stimulates sporing in the latter, it affects both sporulation and growth adversely in the two Aspergilli.

DISCUSSION

The results obtained with *P. griseofulvum* will first be considered. The fungus becomes capable of forming conidia at a very early stage indeed after germination. The attainment of the capacity to form conidia appears, nevertheless, to require the assimilation of exogenous nitrogen and other nutrients. Resting conidia require for germination a nutrient medium, containing at least glucose, nitrate and phosphate, but the nutrient requirements for germination are satisfied by very brief contact with nutrient medium. To develop the capacity for renewed sporulation, the germinating conidium requires, however, at least 12 hr. contact with nutrient medium, with adequate aeration at suitable temperature. This implies a minimal amount of growth in the sense of building new cell material, including proteins, from external nutrients, although the morphological growth involved in the attainment of capacity to spore is scarcely recognizable. It should be noted, however, that the realization of sporing (i.e. after transfer to nitrogen-free nutrient medium) always involves visible vegetative growth before the differentiation of conidiophores and conidia. These observations suggest that the resting conidium is deficient in certain metabolites (possibly enzymes) which must be built up during growth, by the assimilation of exogenous nitrogen, before the fungus is again capable of forming conidia. Once the fungus has passed this 'pre-sporing' stage it remains capable of sporulation, given the required conditions, throughout growth, and certainly for some time after it has exhausted all the available nitrogen in the medium. Probably the vegetative mycelium is capable of sporulation as long as it is viable, although this has not been fully investigated.

Of all the factors inducing sporulation, the most powerful is exposure of the fungus to subaerial conditions. This is not surprising, since such conditions are presumably closely similar to the complex of ecological conditions in which sporulation occurs naturally and to which it is adapted.

Mycelium grown in submerged culture in glucose-nitrate may be induced to sporulate vigorously, at almost any age, by transfer to subaerial conditions, whereas other methods of induction vary somewhat in effectiveness with age of the mycelium. Older mycelium in particular tends to lose the power to respond to added glucose, or to transfer to nitrogen-free medium, whilst still responding by sporulation to subaerial conditions. Subaerial sporulation is little affected by concentration of carbohydrate, presence of calcium or crude glucose, etc., but tends to be delayed if assimilable nitrogen is present, and then to take place when nitrogen assimilation ceases.

The factors which induce sporulation of *P. griseofulvum* in submerged culture fall into three groups. The first group may be considered as having primarily physical effects on the mycelium—presence of initial high concentration of glucose, addition of extra glucose or sorbose during growth, partial temporary desiccation. Reasons have already been given for considering the addition of glucose or sorbose as an osmotic rather than a nutritional effect. Broadly speaking, all these factors have primarily a 'drying' effect on the fungal cells, and seem thus to be directly related to what must be one of the effects of exposure to subaerial conditions.

A second factor is the supply of assimilable nitrogen, which profoundly affects sporulation, although in a complex manner. In general, sporulation tends to be induced when the nitrogen supply is decreased or exhausted, and to be inhibited when nitrogen is added. This again corresponds to the conditions when mycelial branches emerge into the atmosphere, when their nitrogen supply becomes restricted to what is supplied from the submerged parts of the mycelium. Sometimes, however, sporulation is not induced by lack of nitrogen; this is the case with submerged mycelium in normal glucose-nitrate medium, although, remarkably enough, sporulation can be induced by a sudden reduction in nitrogen supply caused by transferring mycelium into the same medium lacking only nitrate. On the other hand, a remarkable feature of the factor in crude glucose is its power to induce sporulation not only at an early stage of growth, when the nitrogen in the medium is still high, but even in conditions when the nitrogen supply is increasing in concentration relative to the other nutrients. This was shown by continuously adding nitrate to a crude glucose medium at a rate faster than it was used by the growing fungus. Sporulation occurred abundantly, and simultaneously, with flasks containing the fungus growing in normal (crude glucose) medium. In spite of these complications, it is clear that the absence of nitrogen is an important factor in inducing, or at least permitting, sporulation to occur. The inhibition of sporulation in induced mycelium by the mere addition of assimilable nitrogen is a phenomenon of considerable significance, which requires fuller investigation.

The third group of factors includes substances (calcium, copper, crude-glucose factor) which specifically induce sporulation in certain conditions. Their effects are most marked in submerged culture and cannot be directly related to aerial conditions. A study of their effects on metabolism might, however, lead to a better understanding of the nature of induction.

The results with submerged culture suggest that the overriding stimulus to sporulation due to aerial conditions may be analysed into at least two broad effects, one associated with a partial desiccation of the mycelium, and the other associated with the absence (or restriction) of exogenous nitrogen. It is interesting that one can begin to resolve a complex ecological effect in this way by physiological experiments. A rather similar complex of physiological conditions has been noted by Méry & Luteraan (1948) in relation to growth habit and production of lipids by fungi. The behaviour of the other species examined is important in this connexion, since there is a certain generality of response (particularly among the various species of *Penicillium*) to the factors which induce sporulation, although the degree of response to a particular factor depends on the species. There is evidence of a general pattern of behaviour elicited, although to a varying degree, by the same set of factors. It should be remembered that the other species were studied in less detail than was *P. griseofulvum*, and that in some cases lack of response may be due to failure to select the effective level of certain factors.

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CONIDIOBOLUS RHYSOSPORUS DRESCHLER IN GREAT BRITAIN

By VIVIENNE J. DRING

Birkbeck College, University of London

(With 1 Text-figure)

A fungus found on apothecia of *Ascobolus* sp. and tentatively identified as *Conidiobolus rhysosporus* Dreschler is described. It produces conidia and zygospores.

Recently a species of *Conidiobolus* was found on apothecia of *Ascobolus* sp. growing on cow dung from a field near Box Hill, Surrey. The affected apothecia showed irregular brown patches on the otherwise greenish yellow surface. In these discoloured areas ascospore discharge was not taking place, but from the surface conidiophores of *Conidiobolus* protruded. A few days later a growth of the fungus was found enveloping an unidentifiable insect larva on the dung.

Discharged conidia were caught on 2 % malt agar plates, and there either formed secondary conidia or germinated to give a mycelium which produced abundant conidia. Later it was found that more vigorous cultures could be obtained on potato-dextrose agar, glucose-peptone agar and oatmeal agar. Again, conidia were found to be produced much more freely in light than in darkness.

After a lapse of 3 weeks the original diseased apothecia, reduced to a brown shrunken condition, were found to contain numerous ridged thick-walled spores bright orange in colour and similar spores also appeared in a few pure cultures kept in darkness. These spores seemed to occur invariably in an intercalary position, but from an examination of the mature structures it was difficult to decide on their mode of origin. However, by following the process from its first observable beginnings, it became clear that fusion of hyphal segments was involved and that the spores were to be regarded as zygospores (Fig. 1 *q*, *r*).

The fungus isolated from *Ascobolus* seems to agree fairly well with *Conidiobolus rhysosporus* isolated and described by Dreschler (1954) from decaying plant material from Louisiana, U.S.A. The conidia are of the right size and shape (20–39 μ (including papilla) × 15–31 μ). Again the zygospores (20–30 μ diameter) agree in size, shape and method of formation. In culture they are formed entirely within the agar. However, the two other types of spore in Dreschler's species: ellipsoidal conidia (not violently discharged) borne on long slender conidiophores, and chlamydospores, have not been seen. So far germination of zygospores has not been observed in the British material. The ellipsoidal conidia described by Dreschler were found only in old cultures where zygospores were germinating. The fungus also agrees in physical characteristics with another of Dreschler's species (*C. osmodes*) but lacks the specific character of that species, namely a smell of benzene hexachloride.

Observations have shown that the fungus is homothallic since abundant zygospores are formed in cultures from a single conidium.

If the discharged conidia are caught on a 2 % malt agar plate and left in the light, most of them germinate directly to give secondary conidia

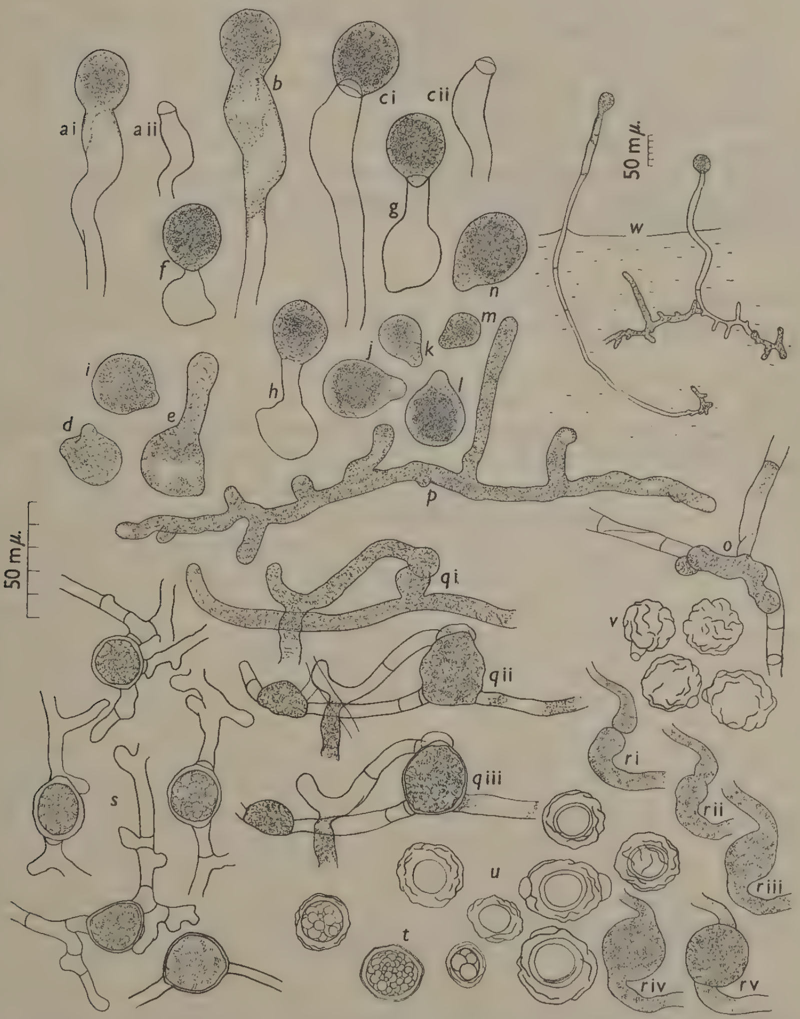

Fig. 1. *Conidiobolus rhysosporus*. (*a–c*) Primary conidiophores and conidia. In *a* and *c* the same conidiophores are shown before (i) and after (ii) discharge. (*d–h*) Secondary conidiophores and conidia in various stages of development. (*i–n*) Discharged conidia. (*o*, *p*) Hyphal segments. (*q*, *r*) Formation of zygospores. (*s*) Five immature zygospores. (*t*) Three nearly mature zygospores. (*u*) Six mature zygospores showing optical section. (*v*) Four zygospores in surface view. (*w*) *In situ* view of hyphae within the agar and condiophores arising from them.

which are discharged on to the lid of the dish (when illuminated from above). When, however, the spores caught on the agar are allowed to germinate in the dark, mycelium production is promoted. The hyphae which form first are very irregular having large numbers of short laterals which may themselves branch but with little increase in length. Nevertheless, branching seems to be primarily in a horizontal plane radiating from the centre of the colony. It is hyphal segments of this kind of mycelium which are concerned with the production of zygospores.

In cultures grown for a few days in darkness and then transferred to light, it can be seen that the irregular hyphae give off straight branches which grow vertically or nearly so. When these hyphae break through the agar surface they are clearly destined to be conidiophores and are positively phototrophic (Fig. 1 *w*).

Cultures of the fungus have been sent to Commonwealth Mycological Institute, Kew, and the Centraalbureau voor Schimmelcultures, Baarn. Herbarium material has also been deposited at Herb. Kew. and at C.M.I. (Herb. I.M.I. 68880).

I wish to express my gratitude to Prof. C. T. Ingold for his help and encouragement, and to Mr P. A. Dixon for collecting the material from Box Hill.

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OBSERVATIONS ON GASTEROMYCETES. VI

THREE BRITISH SPECIES OF *NIDULARIA* FR. SECTION *SOROSIA* TUL.: ECOLOGY AND DISTRIBUTION

By J. T. PALMER

The Hartley Botanical Laboratories, University of Liverpool

(With 2 Text-figures)

Three *Nidularia* species of the Section *Sorosia* Tul. have recently been found in England, where they appear to be common fungi in their respective ecological niches. *N. denudata* Fr. is only otherwise definitely known from two collections in Sweden and one record for Switzerland. *N. duriaeana* Tul. has been collected previously, once in North Africa and in two localities (as *N. castanea* (White) Sacc.) in North America. *N. minutissima* Palmer does not appear to have been recognized before. Related species are discussed.

Introduction

When several collections of *Nidularia denudata* and *N. duriaeana* indicated that the Section might be widely distributed, a methodical survey was made, the localities of the collections being plotted on the Ordnance Survey, using the National Grid Reference system. Time and money have been limiting factors, hence the expeditions in search of *Nidularia* have been made at somewhat irregular intervals, and it is possible that the apparently most promising habitats have been searched more carefully than others. For instance, it was soon realized that well-established clumps or beds of *Juncus effusus* were likely to yield both *N. denudata* and *N. minutissima*, with moorland, bogs and similar acid areas more productive than arable or calcareous places. On the other hand, *N. duriaeana* was collected, at first by chance, on calcareous sand dunes and, so far, has not been discovered elsewhere in Britain.

However, collecting has been as unbiased as possible, with a continuous sampling of both likely and unlikely materials and places. Usually, the finding of a single peridium, or even one peridiole, on a twig or amongst decaying debris has resulted in a more thorough search of the area and, very often, has produced abundant but scattered specimens.

The altitudes of collections have been noted, although they are not considered to be greatly significant. *N. duriaeana* has occurred only on coastal dunes 7·5–15 m. above H.W.L. On the other hand, with a few exceptions, such as low-lying peat mosses at altitudes of about 15 m., *N. denudata* and *N. minutissima* are commonly found at much higher levels, especially in the Pennines, where *N. minutissima* has been collected up to 525 m. and *N. denudata* at a height of 620 m. on Kinder Scout in the Derbyshire High Peak.

The plant substrates were determined according to Clapham, Tutin & Warburg (1952). In all collections on the Soft Rush, the plant appeared to be *Juncus effusus*, but it is possible that some may have been *J. conglomeratus*, which is reported to have a marked preference for more acid soils. *J. inflexus* is recorded as preferring heavy basic or neutral soils.

The species of tree or bush from which twigs or chips originated was usually arrived at by noting what was adjacent and making a comparison but, in a number of doubtful cases, sections were cut and the microscopical structure of the wood examined.

NIDULARIA FR. SECT. *SOROSIA* TUL. (1844)

Nidularia denudata Fr. (1817)

Outside the British Isles, this species has only been recorded a few times: first in Sweden, by E. M. Fries (1817, 1849) on decaying *Alnus* and *Pinus* at Femsjö in 1817 and at Nackanäs, near Stockholm, in June, 1893, but without details of the substratum, by H. Kugelberg (Th. C. E. Fries, 1921); and once in Switzerland, by C. Benzoni (1948), on the decaying branches of a Broom (*Cytisus alpinus*) in *Tilia* forest at Caviano, Canton Ticino, in May, 1942 (see Addendum). Of these, the author has only been able to examine the authentic material of Fries, and the Kugelberg collection, which was apparently seen by Th. C. E. Fries, who also examined the type, does not seem to have been preserved.

N. denudata was first collected in Britain on a rabbit pellet amongst short grass in a disused clay-pit, Brinnington, Cheshire, on 26 September 1953, by Nigel F. Palmer (Palmer, 1957). The gathering consisted of a scattering of peridioles and, on being recognized as a Basidiomycete, obviously a species of *Nidularia*, the area was systematically searched, working out from where the pellet had been found. Sparse collections were made on decaying grass culms, and subsequent searchings revealed it on the decaying leaves of *Juncus effusus*, of which there was a well-established bed in a flooded part of the pit. A fallen branch of *Crataegus* bore developing peridia, whilst a good collection was made on the dead stems of *Rubus*. Brinnington is situated at the foothills of the Pennines in north-east Cheshire at an altitude of 100 m. No special search was made for the species until further accidental collections revealed it on wood and other substrata, particularly in acid districts.

Early experience suggested that well-established beds of *J. effusus* were indicators of probable habitats. Also, *J. effusus*, whilst being extremely common in sour areas, is readily recognized, often at a distance, and produces slowly decaying vegetation in damp situations which can be easily searched. Consequently, a large number of the collections have been on this plant. Other species of *Juncus* were examined, including the ubiquitous moorland *J. squarrosus*, but only *J. inflexus* produced specimens of *N. denudata*. Fallen wood, varying from apparently sound to severely decayed, proved to be the next most productive substratum, with the fructifications developing mainly on the underside in fairly close contact with the earth, or at the junction of the soil and the wood. Only one of the thirty-eight gatherings made on wood was on the bark; the rest were on the decorticated parts of logs, branches, twigs or chips. Whilst lignicolous substrata were occasionally unidentifiable, twelve different kinds of

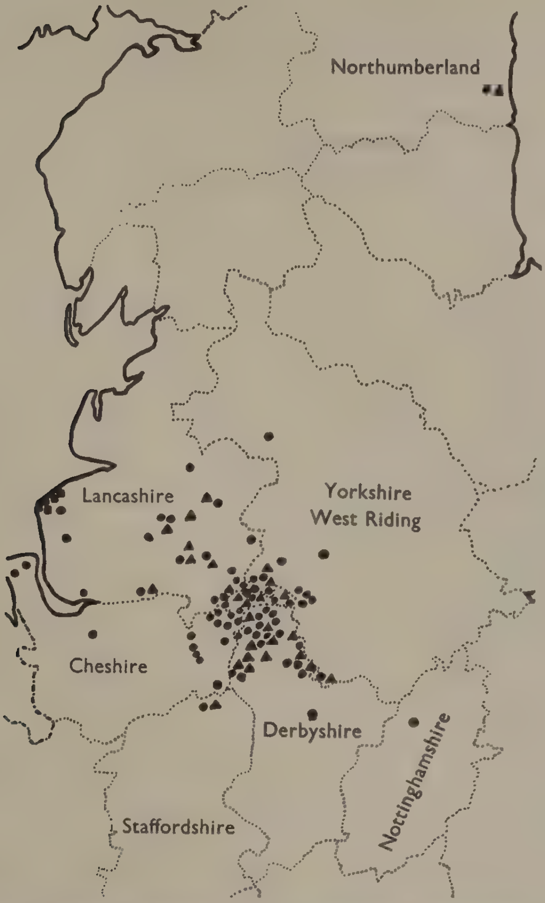

Fig. 1. Distribution in northern England. ●, *N. denudata*: Cheshire (37), Derbyshire (18), Lancashire (24), Northumberland (1), Nottinghamshire (1), Staffordshire (1), and Yorkshire (13) [also Surrey (1)]. ■, *N. duriaeana*: Lancashire (7). ▲, *N. minutissima*: Cheshire (7), Derbyshire (11), Lancashire (10), Northumberland (1), Staffordshire (1) and Yorkshire (8). *Note.* Localities fairly close together have been represented by a single symbol.

wood were determined, of which *Fagus sylvatica* was the most common (Table 1). Interesting collections were those made on the twigs of *Calluna* on open moorland, whilst a surprising gathering was that made on the broken fencing of a rain-gauge station amongst the peat haggs on the Kinder Scout plateau, about the highest point in the southern Pennines. Graminicolous debris was a frequent substratum, with the peridia developing in the damp microclimate at the bases of clumps, frequently in the company of *Sphaerobolus stellatus* Pers. Further collections were made on rabbit pellets, sheep droppings, *Pteridium* and *Vaccinium* debris, unrecognizable plant detritus and undetermined members of the Bryophyta and the Cyperaceae; there was also a single collection on charcoal. All collections had developed under damp conditions and have consisted of both developing fruit-bodies and scattered periodioles. As already mentioned, it was not uncommon to find only scattered periodioles, which are persistent and tend to get washed down into the soil or amongst the *Juncus* roots by the rain.

Table 1. *Substrates of the British collections*

Note. Some collections of *Nidularia denudata* and *N. minutissima* grew on more than one substrate or component of *Juncus effusus*.

Substrate	*N. denudata*	*N. duriaeana*	*N. minutissima*
Ammophila arenaria	.	3	.
Betula sp. (leaves)	.	.	1
Bryophytae	2	.	.
Charred material:			
Bryophytae	.	1	.
Pinus needles	.	1	.
Charcoal	1	.	.
Cyperaceae	1	.	.
Dung pellets:			
Rabbit	4	1	.
Sheep	2	.	.
Graminicolous debris	15	.	1
Juncus effusus			
Dead leaves, etc.	41	.	14
Root stock	.	.	8
Leaf sheaths		.	18
Submerged leaves	.	.	3
Juncus inflexus debris	3	.	.
Plant debris (incl. *Pteridium*)	4	.	.
Vaccineum sp. debris	1	.	.
Wood: (twigs, chips, etc.)			
Acer pseudoplatanus	1	.	.
Alnus glutinosa	1	.	.
Betula sp.	1	.	.
Calluna vulgaris	4	.	.
Crataegus sp.	1	.	.
Fagus sylvatica	12	.	.
Larix decidua	1	.	.
Pinus nigra ssp. *laricio**	.	2	.
P. sylvestris	1	.	1
Quercus sp.	4	.	.
Rubus sp.	1	.	.
Ulex sp.	3	.	.
Unidentified	8	.	.
Total	112	8	46

* Also on needles.

N. denudata has been collected in every month except January and February, when the weather discourages serious collecting, but a piece of wood in the author's garden has produced fresh peridia continuously for practically 2 years. The literature and the author's field observations indicate that other members of the genus, as well as of the family, are definitely seasonal in occurrence and do not appear in Britain until late summer with development ceasing in early winter, although the old peridia tend to persist longer.

The species has been searched for assiduously in calcareous areas such as the Derbyshire dales and the limestone parts of North Wales but without success. A striking instance of its apparent calcifuge habit was noted in a North Derbyshire valley with limestone on one side and gritstone on the other. Several hours searching on the limestone yielded nothing, but the species was collected within 10 min. of moving across to the opposite side of the valley, where acid conditions prevailed. Recently, the author chanced to notice a 'suitable-looking' *J. effusus* bed on the edge of Wimbledon Common, Surrey, and found *N. denudata* in it within 5 min. of commencing his search. A large, well-established bed of *J. effusus* situated a few miles north of Newcastle upon Tyne, produced good material of both *N. denudata* and *N. minutissima* within a similar period.

NIDULARIA DURIAEANA Tul. (1944)

This species was hitherto known only from North Africa and North America. The type collection was made in North Africa by Durieu de Maisonneuve where, according to a note in the Herb. Paris, it grew upon sandy ground amongst stunted moss forming the centre of a sprouting tuft of *Ampelodesmus tenax* Lk. which had been burned the previous year at La Calle, Algeria, in January 1841. In North America, it was redescribed as *Granularia castanea* by White (1902), where Ellis & Everhart found it on a pine-wood roof at Newfield, New Jersey, in May, 1876, as well as in July and September 1883. It was subsequently collected by L. S. Olive (1946) on the under surface of an old piece of rotting canvas, together with *Helicosporium aureum* (Cda) Linder near the Plant Industry Station, Beltsville, Maryland, in May 1945. All these collections have been examined by the author and agree with the British material.

In England, six collections have been made by the author, all being found within a 6 km. stretch of the alkaline dunes from Formby to Ainsdale on the South Lancashire coast and up to 1·5 km. from the H.W.L. with the first being made on the 24 October 1953. The substrata concerned are a rabbit pellet, the dead culms of *Ammophila arenaria*, branches, twigs and dead needles of *Pinus nigra* Arnold ssp. *laricio* (Poir.) Palibin, as well as a patch of charred Bryophytes and pine needles. It was, perhaps, surprising, particularly in such an arid area, that the peridia tended to develop on the upper surface of the substrate, although usually along the line of contact with the sand, where moisture no doubt lingers. Three collections consisted of scattered periodioles alone, and it will be realized as to what difficulties there are in recognizing such a minute species whose periodioles are rarely larger than the frequently coloured neighbouring grains of sand. *N. duriaeana* is probably quite common on the Lancashire dunes, in spite of the dry surroundings. Several other dune areas have been searched but, so far, without success.

NIDULARIA MINUTISSIMA Palmer (1957)

So far as can be ascertained, this species has not been observed previously. It is possible that it has been recorded under one of the uniperidiolar genera such as *Thelebolus*, but no description clearly applicable to it has been seen. Its structure clearly indicates that it is closely related to both *N. denudata* and *N. duriaeana*.

The first collection of this minute species, which produces only uniperidiolar fruit-bodies, *c.* 210 μ diam., was a few scattered peridioles on partly submerged leaves of *J. effusus* at the edge of a pond in a quarry at Coombes, Derbyshire, at an altitude of 300 m. on 5 June 1955. This gathering was so sparse that, although it appeared to be distinctive, it was regarded at the time as an aberrant form of *N. denudata*. The first adequate gathering was made at Kearsley Moor, Lancs, just over 100 m. altitude, on the living root stock and leaf sheaths of *J. effusus* on 13 June 1955. The habitat was rough ground adjacent to a colliery and was covered with scattered clumps of *J. effusus*, which were sampled by pulling up the rush by the roots. It was with surprise that developing peridia and scattered peridioles were found on the root stocks and leaf sheaths. *N. denudata* was present in the same area but was easily distinguished from *N. minutissima*, and this has been found to be true of other gatherings: the two species obviously require similar conditions.

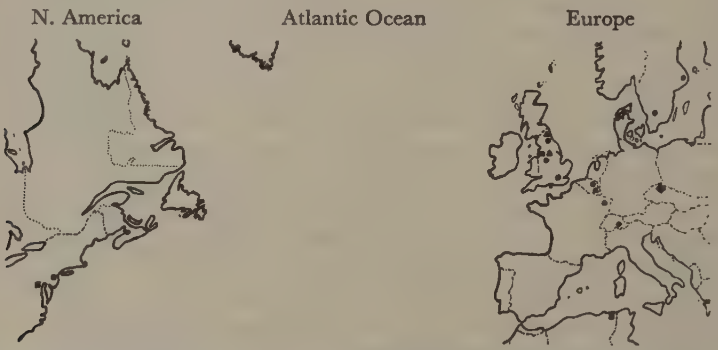

Fig. 2. Geographical distribution of records. ◆, *N. arundinacea*: Czechoslovakia (1). ●, *N. denudata*: Belgium (3), England,* France (1), Sweden (2), Switzerland (1). ■, *N. duriaeana*: Algeria (1), England* and U.S.A. (2). ▲, *N. minutissima*: England.* * See Fig. 1. *Note.* Two of the Belgian collections came from the same locality.

At the time of preparing this paper, thirty-eight collections of *N. minutissima* have been made, mainly in the Pennine area, but with occasional gatherings on low-lying (15 m. altitude) peat mosses, whilst the author collected it on *J. effusus* debris near Newcastle upon Tyne, Northumberland. As with the foregoing species, field work concentrated on elucidating the distribution of the species rather than on increasing the number of collections. All except two collections have been on *J. effusus*, either the root stock and leaf sheaths, occasionally in the company of minute Discomycetes belonging to the Hyaloscyphaceae, or, more rarely, on leaf debris. The other substrata are graminicolous debris, dead *Betula* leaves amongst *J. effusus* debris and a decorticated branch of *Pinus sylvestris* in a dried-up moorland stream bed.

Collections of freshly forming peridia have been made in March and from June to October. Material kept in a damp chamber in the dark has fruited continuously throughout the year. Like *N. denudata*, it probably occurs in all seasons.

This species seems to be the most difficult of the three to collect as its size is almost on the limit of unaided vision. The peridioles are easily brushed off when leaves are removed for inspection, and it seems to require the rather more specialized, if destructive, technique of pulling up the plant by its roots. It is probably quite common, as the Newcastle upon Tyne collection suggests.

Indications are that *N. minutissima* requires slightly more acid conditions than *N. denudata*. A very moist environment appears to be essential, and the peridia seem to develop in a thin film of moisture on the *Juncus* root stocks and leaf sheaths. The finding of scattered peridioles and developing peridia on submerged leaves of *J. effusus* opens the question as to whether this species might not also occur in purely aquatic conditions.

RELATED SPECIES

Two other species belong to this group. *N. fusispora* Massee (1898) was described from a collection by L. Rodway on rotten wood near Hobart, Tasmania, towards the close of the last century. The species is only known from the Type collection and seems to differ from *N. denudata* solely by the shape of the spores. *N. reticulata* Petch (1919) is likewise only known from the Type collection in Ceylon, and differs from the other members of the Section *Sorosia* by the presence of scattered *Bovista* capillitium-like, thick-walled hyphae in the cortex of the peridiole. Berkeley & Broome (1875) recorded the species without details as *N. duriaeana*: the substratum appears to be a piece of banana stem, or some similar vegetable matter.

CONCLUSIONS

Whilst all members of the Section *Sorosia* Tul. have been considered rarities, three species are common to abundant in north-west England, and probably occur elsewhere in the British Isles where the right conditions prevail. Two species, *N. denudata* and *N. minutissima*, have only been found in moderately to highly acid conditions, whilst *N. duriaeana* has so far been collected in England only on calcareous dunes.

N. denudata develops on a wide assortment of substrata whilst *N. duriaeana*, although known from fewer collections, seems to have a similar omnivorous appetite. *N. minutissima* seems to be much more restricted in its requirements, but this may be due to the difficulty of recognizing it in the field. Its frequent occurrence on the sheaths at the bases of living leaves is noteworthy. There is, however, no suggestion that *N. minutissima* is anything but a saprophyte.

A pertinent point is why these species have been found so infrequently. It is understandable, in these days of specialization, that only workers interested in other groups of fungi, the so-called 'micro-fungi', would be likely to find them and, indeed, colleagues, on being shown specimens for the first time, have expressed the view that they probably would have passed them over or would have considered the peridia to be 'spiders' eggs' and the peridioles 'seeds', which they certainly closely resemble.

However, why were they not found by such careful collectors with broad horizons as Berkeley and Cooke? Perhaps it was the fact that moorlands, bogs and sand-dunes were considered to be poor habitats in which to search for fungi.

ADDENDUM

The above paper was read at the First European Mycological Congress in Brussels on 15 September 1956. Subsequently, additional information was received and some further, rather interesting, collections have been made.

N. denudata was collected by the author during the Congress excursions in Belgium in two localities: two distinct collections on *Mollinia* and *Juncus effusus* at Lichtaart, Campine, and on partly buried twigs and chips of *Fagus* and *Quercus* in the Haut-Fays, Middle Ardennes. Both habitats closely resembled British (Eyndhoven *et al.*, 1958).

During 1957, both *N. denudata* and *N. minutissima* were found in moorland situations on *Calluna* twigs and *J. effusus*, respectively, on Foel Fenlli, nr Ruthin, Denbighshire, North Wales (23 June) and Killakee Mountain, Co. Dublin, Eire (15 Sept.), where *N. denudata* also occurred on sheep pellets, and both species were collected on *J. effusus* at Glendalough, Co. Wicklow, Eire (18 Sept.). In England, *N. denudata* has now been collected down to the south Lancashire coast where, for the first time, it was found in the damp slacks on bryophytes, a rabbit pellet, *Pinus nigra* and *Populus* twigs, and a collection on graminicolous debris on dune pasture at Formby was within 20 cm. of typical *N. duriaeana* on the same substratum. *N. duriaeana* was also recollected upon most of the recorded substrata with the addition of *Populus alba* twigs, and a new, more southerly dune station was Hightown. A most interesting collection of *N. minutissima* was on dune pasture at Formby, where the species was scattered but abundant on dead stems, etc., in a 12 cm. deep, well-established bed of *Polytrichum formosum*. The dune substrates for all three species gave strongly acid reactions.

During the Congress, Mons. G. Malençon informed the author that Prof. René Maire determined macroscopically as *N. denudata* a collection which they jointly made at the base of a dead clump of a *Juncus* sp. in a swampy area near Lunéville (Meurthe & Moselle), France, in September 1936. The material was not retained.

Hjortsberg (1954) listed two collections of *N. denudata* for Piparböle (30 Sept. 1951) and Stöcksjö (4 Nov. 1951), Umeå, which were so determined by Dr Seth Lundell. The writer has examined the first collection, preserved in Herb. Uppsala, and considers it to be *Nidularia farcta* (Roth. ex Pers.) Fr. From correspondence with both Herr Hjortsberg and Dr Lundell, it is probable that the Stöcksjö material is not *N. denudata*.

Unfortunately, it was not possible to establish contact with Sig. Carlo Benzoni, who (1948) recorded *N. denudata* for Switzerland. Inquiries made through the medium of Dr Meinhard Moser have indicated that the Caviano collection was lost whilst being returned from an exhibition by an Italian society.

N. arundinacea Velenovský (1939), recorded on the withered leaves of *Phragmites* at the edge of a lake near Božkov, Czechoslovakia, is a further member of the Section. The author has examined the type and, whilst he considers it to be probably an immature *N. denudata*, in view of the sparsity of the material, he has proposed (Palmer, 1958) to leave the name undisturbed until it has been found again.

A collection ex Herb. E. Boudier on woody debris in Herb. Paris, determined as *N. denudata* and labelled 'Lyon in Caldariis Dr Riel', closely agrees with *N. reticulata* Petch and probably represents an introduction from the tropics.

The author is indebted to the following who have assisted him in various ways: Mr Winckworth Allen (Dublin), Mr S. S. Bates (Stalybridge), Dr R. W. G. Dennis (Kew), Prof. Rob. E. Fries (Stockholm), Prof. Roger Heim (Paris), Herr L. Hjortsberg (Umeå, Sweden), Dr Seth Lundell (Uppsala), Mons. G. Malençon (Rabat, Morocco), Dr M. Moser (Tirol), Prof. J. A. Nannfeldt (Uppsala), Prof. L. S. Olive (New York), Dr A. Pilát (Prague), Dr D. P. Rogers (New York) and Dr E. M. Rosser (Manchester).

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Trans Brit. mycol. Soc. **41** (1), 64 (1958).

BRITISH RECORDS, 17–20

17. **Asteromyces cruciatus** Moreau (*Rev. Mycol.* **6**, 50–94, 1941). In open sand approximately mid-way between high- and low-water marks of ordinary tides, Studland, Dorset, February 1953; Sandwich, Kent, April 1953, leg. J. C. Brown, det. Dr M. B. Ellis, Herb. I.M.I. 60913. A species which appears to be tolerant of high concentrations of sodium chloride (see Moreau, loc. cit.; Brown, 1957, Ph.D. Thesis, University of London).

JULIET C. BROWN, *Royal Holloway College, University of London*

18. **Unguiculella falcipila** v. Höhn. (*Sitz. Akad. Wiss. Wien*, **115**, pp. 654, 1281, 1906). This minute Discomycete was first found here in June 1953 by E. St A. Glynn in rather scant quantity on dead *Urtica* stems at Datchett, Bucks. It seems to be intrinsically rare for, despite continual search meanwhile, it was not found again until June 1956 when it occurred in abundance and in fine condition on *Urtica* stems at Siddington, Cheshire. Microscopically it is at once known by its Hyaloscypha-type hairs, of which many are strikingly curved in the shape of a sickle. Herb. W.D.G. 1097 and Herb. Kew., Paris and Uppsala. I use the epithet *falcipila* because our fungus is surely identical with that described by von Höhnel which also was on *Urtica* stems. If, as von Höhnel asserts (loc. cit. p. 1280), this fungus is identical with *Pezizella hamulata* Feltgen (*Vorstud. Pilz. Luxemb. Nachtr.* **3**, p. 51, 1903) the epithet *hamulata* has priority. Feltgen's fungus was on stems of *Trifolium*—a host so different as to leave doubt as to the identity of the fungi. In any event the epithet *hamulata* had a far earlier use for *Helotium hamulata* Rehm (Winter in *Hedwigia*, p. 56 1881) a distinct and much larger fungus of *Cirsium* stems, which von Höhnel transferred to *Unguiculella* as *U. hamulata* (Rehm) v. Höhn. (*Sitz. Akad. Wiss. Wien*, **127**, p. 610, 1918).

W. D. GRADDON, *Congleton, Cheshire*

19. **Helotium geogenum** Cooke (Dennis in *Mycol. Pap. C.M.I.* no. 62, p. 71, 1956). About two dozen specimens scattered over a few square yards of bare soil on steep wooded hillside at Richmond, Yorks, September 1956. Spores $26–31 \times 4{\cdot}5–5\ \mu$. Herb. W.D.G. 1129.

W. D. GRADDON, *Congleton, Cheshire*

20. **Helotium laetum** (Boud.) Sacc. (Dennis in *Mycol. Pap. C.M.I.* No. 62, p. 91, 1956). A beautiful ochraceous-golden species growing in dense colonies on very wet woody substrate. Twice collected in September 1956 (*a*) Yarncliff wood, Longshaw near Sheffield, with spores, $18{\cdot}5–19{\cdot}5 \times 5–5{\cdot}5\ \mu$ (Herb. W.D.G. 1119) and (*b*) near Richmond, Yorks, with spores $18–21{\cdot}5 \times 4–5{\cdot}5\ \mu$ (Herb. W.D.G. 1150).

W. D. GRADDON, *Congleton, Cheshire*

Trans. Brit. mycol. Soc. **41** (1), 65–73 (1958).

STUDIES IN THE DEVELOPMENT AND CYTOLOGY OF *PUCCINIA PENNISETI* ZIMM.

By U. K. KULKARNI

Maharashtra Association for the Cultivation of Science,
Law College Buildings, Poona 4, India

(With 5 Text-figures)

This paper reports the development and cytology of the pycnial and aecial stages of *Puccinia penniseti* on *Solanum melongena* and the uredial and telial stages on *Pennisetum typhoides*. The rust fungus follows the normal pattern, as reported for many other macrocyclic rust fungi, except in the formation of 2-celled basidium and quadri-nucleate basidio spores. The number of nuclear divisions in the basidium and basidiospores is three instead of two resulting in eight daughter nuclei instead of four.

Puccinia penniseti Zimm. is a Eu-type, macrocyclic, heteroecious rust and causes leaf rust of Bajra (*Pennisetum typhoides*) in the tropics. The disease cycle, the morphology of the spore forms, and the alternate host have been reported by Ramakrishnan & Soumini (1948). The fungus produces its pycnial and aecial stages on the egg plant, *Solanum melongena*, and its uredial and telial stages on *Pennisetum typhoides* (see Fig. 1). The development and cytology of the various spore forms have not so far received attention and the formation of a two-celled basidium and quadri-nucleate basidiospores by this fungus, as recently reported by the writer (Kulkarni, 1953), made a study of the subsequent nuclear development especially necessary. The results of such an investigation are reported in the present paper.

MATERIALS AND METHODS

Puccinia penniseti Zimm. provided excellent material for a detailed cytological study, on account of the ease and success with which the host plants could be inoculated experimentally. Successful infections were obtained on *Solanum melongena* and *Pennisetum typhoides* with the telial material collected in nature and stored at room temperature (26–27° C.). The young leaves of *Solanum melongena* (grown in pots) were inoculated with fresh basidiospores obtained from germinating teliospores of *Puccinia penniseti* by means of a camel-hair brush and incubated under the bell jar for 48 hr. The leaves of *Pennisetum typhoides* were inoculated with spore-suspension of aeciospores by means of an atomizer. The inoculated leaves showing successful infection were fixed daily in F.A.A.* They were then embedded in paraffin and 6–10 μ sections cut and stained in Heidenhain's hematoxylin, counterstained with Orange G.

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* Formalin (40%) 7 ml., glacial acetic acid 7 ml. and 50% alcohol 100 ml.

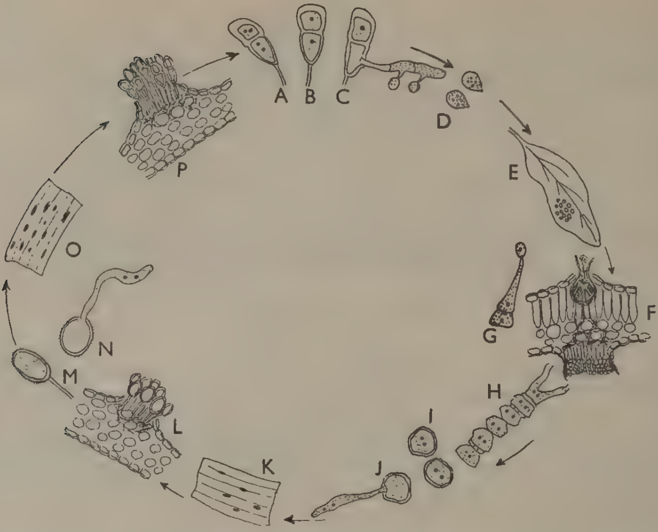

Fig. 1. Diagrammatic nuclear life cycle of *Puccinia penniseti* Zimm. A, young binucleate teliospore; B, mature teliospore with fusion nucleus; C, teliospore germinating; D, quadrinucleate basidiospores; E, leaf of *Solanum melongena* with aecia; F, T.S. of same showing pycnium (on the upper side) and aecium (on the lower side); G, pycniophore with pycniospore; H, chain of aeciospores and intercalary cells arising from two-legged basal cell; I, mature binucleate aeciospores; J, germinating aeciospore with binucleate germ tube; K, leaf of *Pennisetum typhoides* with uredia; L, T.S. of same showing uredium; M, mature urediospores; N, germinating urediospore with binucleate germ tube; O, leaf of *P. typhoides* with telia; P, T.S. of same showing telium.

RESULTS

Pycnium and pycniospores (Fig. 2)

Development. The basidiospore germ tube penetrates the leaf directly through the cuticle and gives rise to primary multicellular hyphae, which are regularly uninucleate (gametophytic mycelium). The hyphae grow between palisade cells of the host (Fig. 2*a*) and force their way between epidermal and palisade layers. These subepidermal hyphae branch rapidly and form a continuous mat of mycelium with a layer of polygonal cells at the top (Fig. 2*b*). The growing mycelium tends to converge from all sides towards a central point (Fig. 2*a*, *b*), where the pycnial initial develops. The mycelium now breaks up and forms a pseudoparenchymatous tissue made up of polygonal cells and surrounds the entire host cavity, where ultimately the pycnium is located. The epidermis covering the pycnium is cut off from its natural food supply from the rest of the leaf tissue and is under tension of the growing pycnium. The weakened epidermis breaks under the growing pressure of buffer cells from within (Fig. 2*c*) and gives rise to an ostiole. Pycnia develop within 5–6 days. The paraphyses are absent in the early stage but appear as long, straight bristles at the mouth of the ostiole as the pycnium attains maturity (Fig. 2*d*). Pycniospores are oblong or elliptic, containing a single haploid nucleus and are produced on simple basal pycniophores lining the inner wall (Fig. 2*d*).

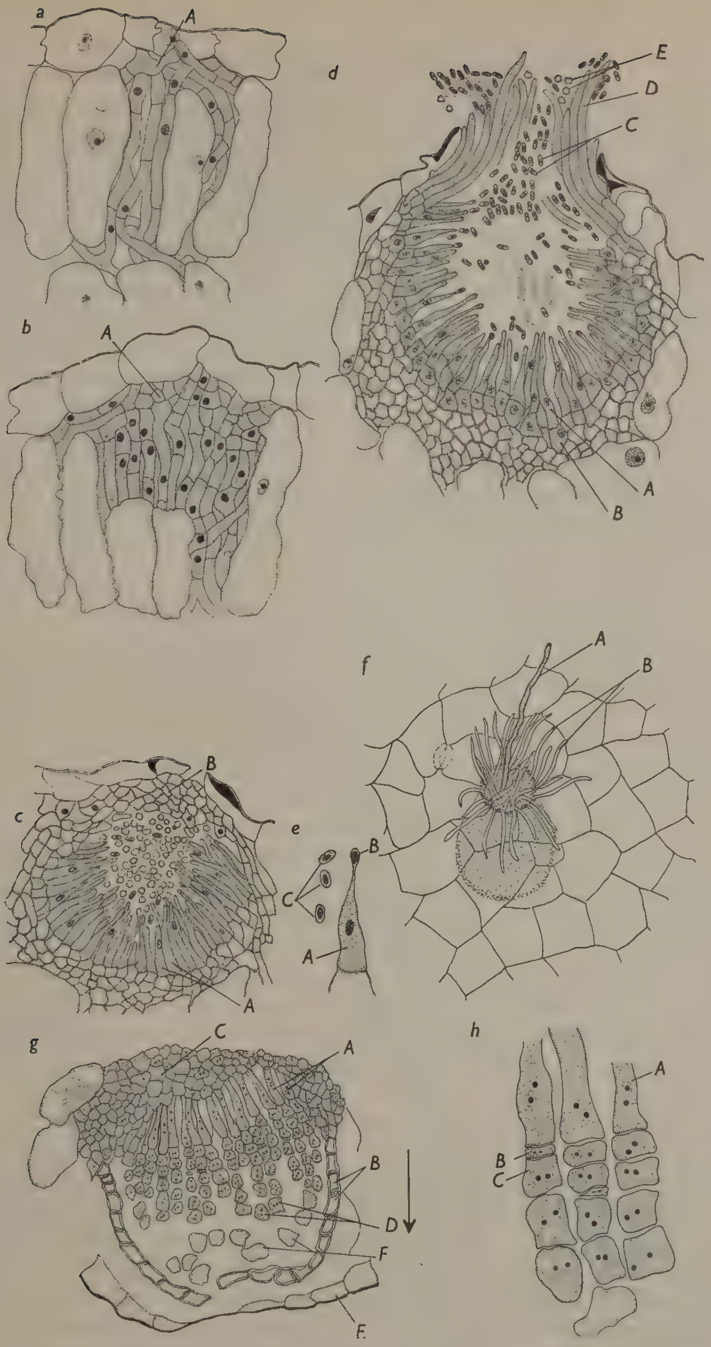

Fig. 2. (*a*) Gametophytic mycelium resulting after penetration of basidiospore, between palisade tissue and epidermis, × 350. (*b*) Same later stage, × 350. (*c*) Formation of young pycnium showing young pycniophores *A*, and buffer cells *B*, × 350. (*d*) Mature pycnium showing pycniophore *A*, stalk cells *B*, pycniospores *C*, paraphyses *D*, and buffer cells *E*, × 350. (*e*) Mature pycniophore *A*, producing a terminal pycniospore *B*, and mature uninucleated pycniospores *C*, × 570. (*f*) Surface view of a pycnium as seen under microscope showing the flexuous hyphae *A* and paraphyses *B*. (*g*) Aecium showing binucleate basal cells *A*, peridium *B*, pseudoparenchymatous cells *C*, binucleate aeciospores *D*, epidermis of the host *E* and buffer cells *F*, × 155. (*h*) Binucleate basal cells with chain of binucleate aeciospores *C*, and intercalary cells *B*, × 570.

Flexuous hyphae were observed in free-hand sections (Fig. 2*f*) but not in microtome sections, due probably to their being dislodged in the process of staining.

Cytology. The gametophytic hyphae between the epidermal and palisade layers are multicellular, each cell being uninucleate (Fig. 2*a*). The nuclei in the pseudoparenchyma layer, from which short branches bearing pycniophores arise, are similar to those of the vegetative mycelium. The young pycniophores are thick and often curved and taper to a rather blunt tip (Fig. 2*c*). They contain dense cytoplasm and a single nucleus, which divides and the two daughter nuclei are separated by a septum into a short stalk-cell (Fig. 2*d*) and a terminal cell, which develops into the pycniophore proper (Fig. 2*d*). The nucleus in the pycniophore divides into two daughter nuclei one of which migrates into the developing pycniospore (Fig. 2*e*). The basal nucleus divides again, and the process is repeated giving rise to a new pycniospore in its place.

Aecium and aeciospores (Fig. 2)

Development. In the middle layer of an infected brinjal leaf, wefts of hyphae appear which after diploidization are destined to form the aecial spore-beds. These wefts of hyphae together with a mass of pseudoparenchymatous cells form the aecial primordium, which is made up of (1) basal cells (Fig. 2*g*) and (2) pseudoparenchymatous cells (Fig. 2*g*). The basal cells are fertile and take part in the production of chains of aeciospores; while the pseudoparenchymatous cells are sterile and give rise to the peridium (Fig. 2*g*). After peridial formation, the fertile basal cells (Fig. 2*h*) divide into unequal cells, the larger forming the aeciospore proper, the smaller the intercalary cell (Fig. 2*h*). The repeated division of the basal cells and nuclei ultimately give rise to a row of aeciospores and intercalary cells alternating with each other (Fig. 2*h*).

Cytology. The multicellular gametophytic (uninucleated) mycelium becomes sporophytic (binucleated) by the process of diploidization (cell-fusion). The binucleate fertile basal cells ultimately divide producing the binucleate aeciospores and the binucleate intercalary cells (Fig. 2*h*). The nuclei of the aeciospores soon become completely reorganized and those of the intercalary cells degenerate.

Germination and viability of aeciospores. Aeciospores germinate in hanging-drop cultures of sterilized water in 3–4 hr., producing a simple or branched germ tube, forcing its way through the exospore, where it becomes constricted. The germ tube is vacuolate in the lower portion and densely granular at the tip. The aeciospores were found to retain their viability for 20–30 days in dry leaves stored at laboratory temperature (25–27° C.).

Uredia and urediospores (Fig. 3)

Successful infections of leaves of *Pennisetum typhoides* were obtained by inoculation with fresh urediospores as well as aeciospores; the spore suspension was atomized over moist leaves, which were incubated under a bell jar for 48 hr. Typical chlorotic areas appeared in 5–6 days and the necrotic pustules in 8–10 days.

The germ tube of the urediospore penetrates the leaf through the stoma and produces hyphal cells in the substomatal cavity; the cells are more or less cylindrical and regularly binucleate; this is the uredial initial (Fig. 3*a*). The cells now divide repeatedly, producing a compact tissue of binucleate cells and give rise to the uredial basal cells or primordium (Fig. 2*b*); these primordial cells elongate vertically producing the spore-initial (Fig. 3*d*) by the formation of two unequal binucleate cells through the process of conjugate division of the original nuclear pair (Fig. 3*b* (A)). The upper

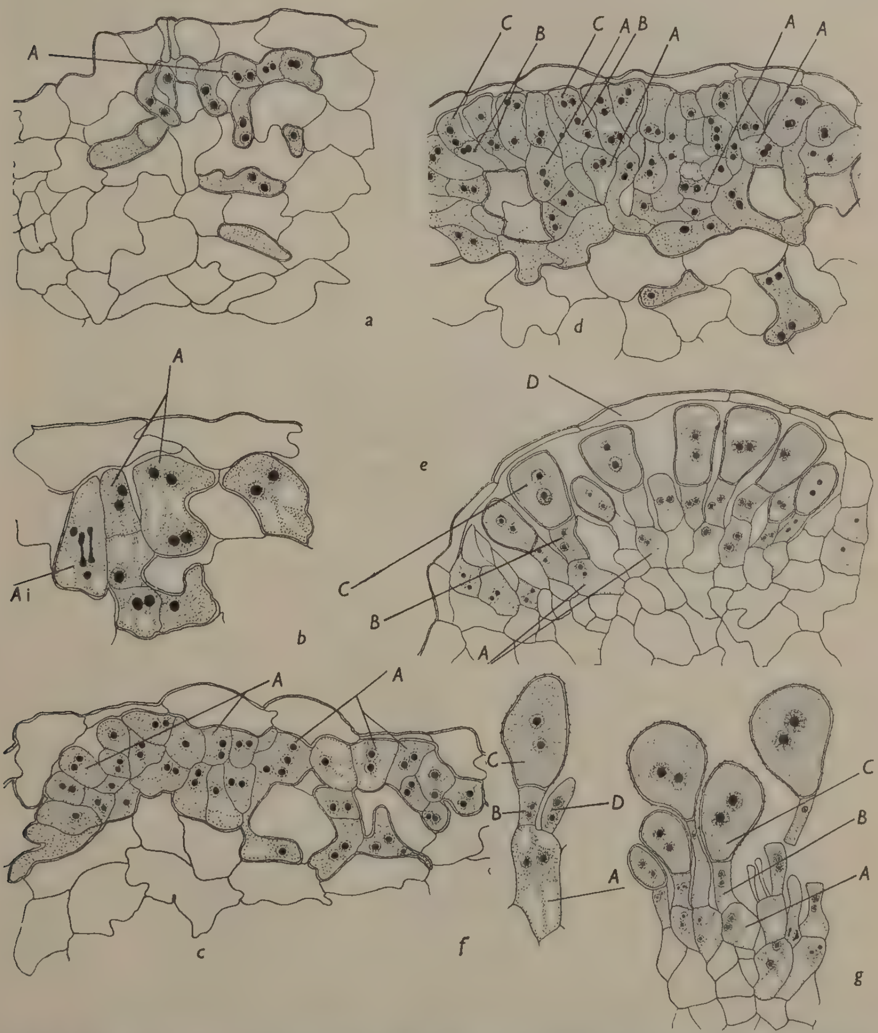

Fig. 3 (*a–g*). Stages in the development and nuclear behaviour of uredia and urediospores. *A*, basal cells; *B*, stalk cell; *C*, uredial mother spore; *Ai* conjugate nuclear divisions. *D*, lateral bud giving rise to urediospore initial. (*a, c, d, e, g,* × 525; *b, f,* × 850.)

cell becomes the urediospore (Fig. 3*e* (C)) and the lower a stalk-cell (Fig. 3*e* (B)). Spore-initial in the form of lateral bud arising from basal cells has been also occasionally noticed (Fig. 3*f* (*D*)). The above process is repeated several times, the nuclear pairs are reorganized, ultimately giving rise to the fully-fledged pedicellate urediospores comprising the fully developed uredium (Fig. 3*g*).

Germination and viability of urediospores. Fresh urediospores sown in sterilized water in hanging-drop cultures, readily germinate through the

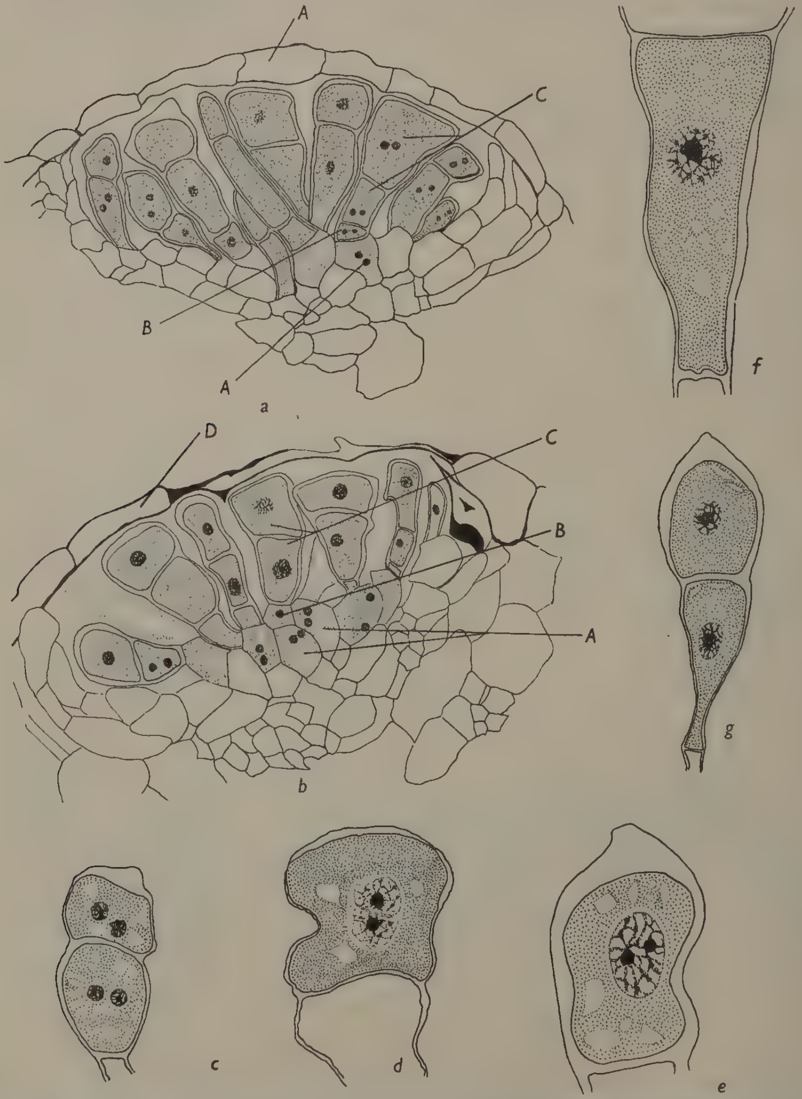

Fig. 4 (*a–g*). Stages in the development and nuclear behaviour of telia and teliospores. *A*, basa cells; *B*, stalk cells; *C*, teliospore mother cells; *D*, peridium. (*a*, *b*, × 525; *c*, *g*, × 850; *d*. *e*. *f*. × 1525.)

germ pores in 3–4 hr., producing a germ tube with a vacuolate lower portion and densely granular tip; branching of the germ tube occurs with high humidity. The urediospores remain viable under laboratory conditions (25–27° C.) in dry leaves for 30 days, after which the spores show rapid deterioration.

Telia and teliospores (Fig. 4)

The telium develops either in the old uredia or independently as a new structure, from the binucleate basal cells (Fig. 4*a*, *b*), the process being similar to that described for uredia. The binucleate basal cells undergo division and cut off vertical rows consisting of three cells (Fig. 4*b* (*B*, *C*)) which are regularly binucleate. The upper two cells form the 2-celled teliospore and the lower one develops into a stalk. The teliospore is binucleate in the initial stages (Fig. 4*c*), the conjugate nuclei, however, undergo a change and ultimately fuse in the mature teliospore (Fig. 4, *c*–*e*). The two nuclei press on each other, the surrounding membrane disappears

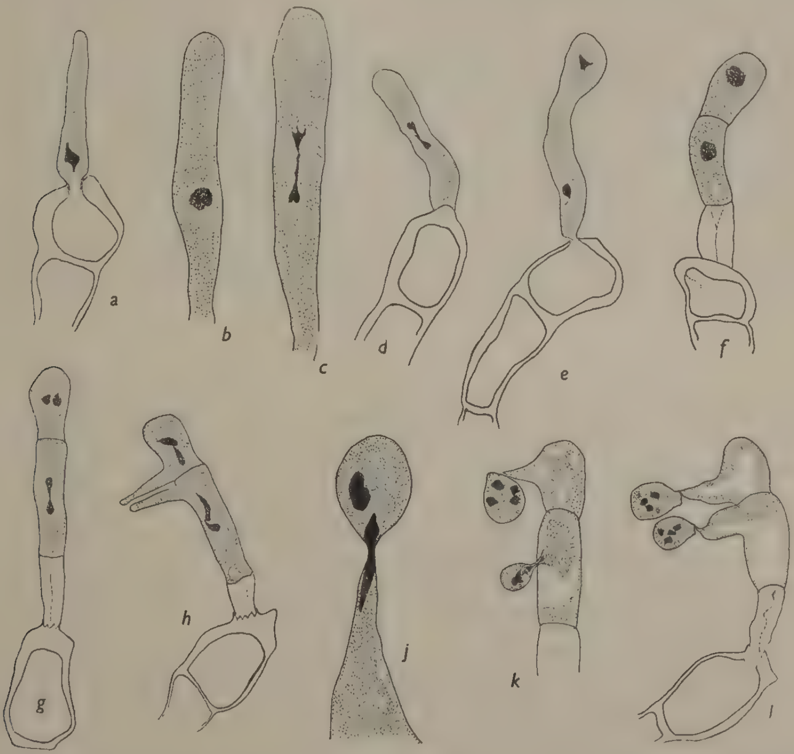

Fig. 5 (*a*–*l*). Stages in the germination and nuclear behaviour of teliospores. (*a*, *b*) Migration of the fusion nucleus into the basidium. (*c*–*e*) Division of the fusion nucleus in the basidium. (*f*) Development of cross septa in the basidium. (*g*) Second nuclear division in the basidium. (*h*) Initiation of sterigmata and nuclear division. (*j*) Migration of the daughter nuclei from the basidium into the basidiospores. (*k*, *l*) Quadrinucleate condition of the basidiospores. (*a*, *d*–*h*, *k*, *l*, ×525; *b*, *c*, ×850; *j*, ×1525.)

at the point of contact, ultimately allowing them to come together, and after developing a common membrane (Fig. 4*e*) the fusion nucleus becomes organized as a diploid nucleus (Fig. 4*f*).

Germination and viability of teliospores. The teliospores germinate 48 hr. after a 3–5 month period of rest. Fresh teliospores have not been found to germinate. In general, only one cell of the spore puts out a basidium, although both cells have been observed to germinate in a few cases. After the basidium has attained some length, the fusion nucleus from the spore-mother-cell migrates into the young basidium and occupies a central position (Fig. 4*a*); the fusion nucleus in the basidium now prepares for division and ultimately gives rise to the binucleate condition, one nucleus migrating to the base and the other to the apex of the basidium (Fig. 5, *c–e*). A brief account of the process of germination and nuclear behaviour has already been given by the writer (Kulkarni, 1953). The two nuclei are separated by a cross-wall and undergo further division; the basidium remains two-celled, each cell putting out a sterigma, usually near the cross-septum, the nuclei again divide, developing a binucleate condition in each of the two cells of the basidium (Fig. 5*f*, *g*); the apex of the sterigma develops into an ovoid cell and the two daughter nuclei from the basidial cells migrate into the apical basidiospore by streaming through the narrow canal joining the sterigma with the basidiospore (Fig. 5*h*, *j*, *l*); the two basidiospores are initially binucleate, and finally undergo further division giving rise to the quadrinucleate condition of the mature basidiospore (Fig. 5*l*). The teliospores thus produce a 2-celled basidium and quadrinucleate basidiospores, as a result of three divisions of the original fusion nucleus instead of two, as in normal rust life cycles.

The period of viability of the teliospores was tested on dry material by periodical germination studies. The spores remained viable for 6–7 months at laboratory temperature (25–27° C.).

Discussion

It will be observed from the results obtained that the nuclear behaviour of this rust, in respect of the two spore forms on the aecial host, follows in general, the pattern reported by Allen (1930) for *Puccinia graminis*. The parasitic mycelium within the host resulting from the infection of quadrinucleate basidiospores is regularly uninucleate and this condition persists until it reaches the aecial primordium, where the binucleate condition or dikaryon is initiated by cell fusion (Christman type) (Kulkarni, 1956). Unfortunately, the earlier stages of infection and penetration by the quadrinucleate basidiospores have not been observed. Evidently the original supernumerary nuclei degenerate to give a uninucleate parasitic mycelium. The development of the aecium, the aecial chains and aeciospores, together with the mode of dikaryotization, follow a line of development closely allied to *Cronartium ribicola*, as described by Colley (1918).

The development and cytological behaviour of the uredium follows a normal pattern. These processes, however, exhibit several abnormal features in respect of teliospores, the formation of the basidium and basidiospores. No four-celled basidia, so usual with macrocyclic rusts, have been observed in this rust, thus contradicting the report by Jackson (1935), that the production of two-celled basidium is associated with microcyclic rusts. *P. penniseti*, however, has been found to be macrocyclic, without showing any tendency for modifications in its normal heteroecious nature. It may also be noted with interest that the fusion nucleus in the basidium undergoes three successive divisions instead of two, reminiscent of the nuclear behaviour in many Ascomycetes, finally resulting in eight daughter nuclei, distributed evenly into the two basidiospores.

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COLONIZATION OF ROOTS OF *PISUM SATIVUM* L. BY FUNGI

By H. STENTON

Department of Botany, University of Hull

The colonization by fungi of the first 4 cm. (measured from the cotyledon insertion) of tap root of *Pisum sativum* was investigated, over a period of 87 days, by a washing technique which permitted the isolation of active root surface fungi. Over 40 different fungi were isolated but only the genera *Cylindrocarpon*, *Pythium*, *Fusarium*, *Gliocladium* and *Mortierella* were abundant. Most 2 mm. sections of root yielded only one fungus, but the percentage of sections with two fungi increased with age. Colonization was patchy at first, areas of occupied and vacant surface alternating. Lateral spread was rapid but occupied areas were not readily invaded by a second organism. The rate of colonization was much slower in winter than in summer.

INTRODUCTION

The study of the fungal flora of the soil has now reached a stage where broad floristic surveys can contribute little new information. As Garrett (1955) and Stanier (1953) have pointed out, the microcosm of the soil presents an infinite number of micro-habitats for fungi and other soil organisms. It is with the factors which govern the colonization and degradation of these substrates that the soil microbiologist who is concerned with ecological problems must now deal. The successful study of these problems requires that new techniques, which permit the examination of precisely defined substrates, should be developed. The selection of suitable substrates presents many problems. It is essential that the substrate should be easily recognizable, that it should be readily recoverable from the soil, that its age should be known and that it should be able to withstand manipulation after it has been retrieved from the soil. The surface of living roots is a substrate which fulfils many of these requirements. For this reason an attempt has been made to study the fungi that colonize the roots of *Pisum sativum*. It was found that if surface-sterilized pea seeds were germinated on moist, sterile filter-paper in Petri dishes the radicles, in most cases, remained free from fungal mycelium for at least 7 days. It thus appeared that the surface of the pea root would present a virgin ecological niche to soil micro-organisms. Furthermore, the continued meristematic activity of the root apex would provide a continual supply of ecologically open surface.

METHODS

Pea seeds were surface-sterilized by shaking in Nance's solution (1 g. $HgCl_2$, 5 ml. 'Teepol' in 1 l. distilled water) for 15 min., followed by washing with several changes of distilled water. Washing was continued until no further foaming of the detergent in the Nance's solution occurred. The seeds were planted, either in the author's garden or in pots of the garden soil in the glasshouse, depending on the season. At intervals, batches of the seedlings were sampled as follows. The bulk of the soil was carefully removed and the root system was cut off just below the insertion of the cotyledons. The first 4 cm. of tap root below the cotyledon insertion was retained for analysis of the root surface flora. In young roots this might include the root apex. The cotyledon insertion was taken as the point of reference since it could be recognized even on old plants, the cotyledons of which had long since disappeared. Furthermore, owing to the age gradient from the cotyledon insertion downwards, age could be estimated precisely only in the uppermost portion of any root. For instance, if the peas were examined 21 days after sowing, only a small portion of the main root would be approximately of that age. The rest of the root system would have an age gradient down to zero days at the absolute tip. After removal of laterals, each root length was placed in a fine gauze sieve and individually washed free from soil, by means of a fine jet of water. Sometimes it was necessary to loosen firmly fixed particles of soil by rubbing them gently with the tip of the glass jet. Approximately 3 l. tap water was sufficient to wash five root lengths. Root nodules were trimmed off the root surface during the preliminary washing, for two reasons. First, many nodules have a very restricted point of attachment, so that most of the nodule is inevitably lost when the root is cut into short sections. Secondly, when a nodule is so cut, the bacteria are liberated on to the washed root and may suppress the development of fungi on the isolation plates. If the nodule is cut off prior to washing, many of these bacteria are removed.

After washing, the root lengths were shaken in ten changes of 50 ml. sterile streptomycin sulphate solution (51·8 i.u./ml.) in 250 ml. conical flasks; not more than three root lengths were placed in each flask. The first shaking lasted 5 min. and subsequent shakings were for 2 min. each. Each washed root length was now cut into twenty 2 mm. sections, taking all practicable precautions to avoid contamination, and the sections were plated on Czapek-Dox + 0·5 % yeast-extract agar. Four sections were placed in each dish and the position of each section with respect to the cotyledon insertion was noted. Streptomycin sulphate (51·8 i.u./ml.) was added to the culture medium to suppress bacteria. It appeared to make little difference if the antibiotic was added to the culture medium prior to sterilization or if it was sterilized separately by filtration and added aseptically to the cooled, melted agar just before the plates were poured. The plated root sections were incubated at 25° C. for 2–3 days and then kept on the laboratory bench for up to 1 month. They were examined after 3–4 days and the presence or absence of fungi was recorded. The examination was repeated at intervals of a few days until the plates were discarded. The identity of the fungi was noted as it became apparent, and representative isolations were made.

Results

More than 40 different fungi were isolated. Many occurred infrequently and no assessment of their role in the colonization of the virgin root surface has been attempted. Table 1 gives the distribution of the most frequently occurring fungi on the upper and lower halves of the root lengths, and Table 2 records the size of samples on which these observations are based.

Table 1. *List of most frequently occurring root surface fungi*

	No. times recorded		
Fungus	Upper 2 cm.	Lower 2 cm.	Total
Mortierella	36	37	73
Mucor	24	3	27
Pythium	73	46	119
Acrostalagmus cinnabarinus	6	6	12
Cephalosporium	7	3	10
Cylindrocarpon	305	330	635
'Dematiaceae'	22	26	48
Fusarium	154	124	278
Fusarium, blue	26	6	32
Fusarium, red	21	24	45
Fusarium, white	53	40	93
Gliocladium roseum	78	29	107
Unidentified	22	26	48

Only five genera were abundantly represented on the root surfaces. Phycomycetes were represented by *Pythium* and *Mortierella*. The other genera were all Fungi Imperfecti. *Cylindrocarpon* was by far the most abundant and constantly occurring root-surface fungus. *Fusarium* was also very common; this genus has been treated as a whole and, in addition, three of the more easily recognizable species have been analysed separately. *Gliocladium roseum* was an interesting example of a late colonist.

Table 2. *Summary of data on which results are based*

Age of roots (days)	7	14	21	28	37	43	50	87	Total
No. root lengths	23	11	9	5	5	5	5	5	68
No. 2 mm. sections	427	220	178	100	100	100	100	100	1325
No. sections without fungi	81	—	—	—	—	—	—	—	—
% colonized	94	—	—	—	—	—	—	—	—

Speed and extent of colonization of the root surface by fungi

For analysis the data have been divided into two groups, those appertaining to the upper 2 cm. root surface and those appertaining to the lower 2 cm. root surface. Comparisons can then be made on the basis of age of root surface (i.e. the upper 2 cm. is older) and time of exposure to colonization by fungi (Table 3).

Lower 2 cm. root surface. At 7 days only 20% of the root surface was uncolonized. The subsequent colonization of this final 20% was quite slow and it was only after a further 36 days that the amount uncolonized was reduced by half to 10%. At 87 days the entire surface was colonized.

Upper 2 cm. root surface. Here the rate of colonization was considerably faster. At 7 days 29 % was uncolonized but this had been reduced by half to 15 % after a further 7 days. At 37 days the entire surface was colonized.

Table 3. *Percentages of root sections in the various age groups yielding* 0, 1, 2 *or* 3 *colonies*

Age of roots (days)		7	14	21	28	37	43	50	87
0 colonies	U	29	15	8	4	0	0	0	0
	L	20	14	15	16	8	10	10	0
1 colony	U	60	68	66	76	56	74	82	42
	L	76	75	74	66	78	58	78	58
2 colonies	U	10	12	22	18	42	20	18	54
	L	5	11	11	16	14	28	10	36
3 colonies	U	4	4	4	2	2	6	0	4
	L	5	0	0	2	0	4	2	6

U = upper 2 cm. root surface; L = lower 2 cm. root surface.

Other information concerning the pattern of colonization of the root surface may be obtained from the data in Table 3. For instance, the majority of root sections yielded only a single colony. Furthermore, there was no marked decrease in the percentage of single colonies with age. This indicates that once a portion of root surface has become colonized by a fungus it is not readily invaded by other fungi. One must not, however, overlook the fact that the selective action of the isolating medium may have influenced these results. A much smaller percentage of root sections yielded two colonies after being plated. Two points should be noted in this connexion: (1) at all ages, except 43 days, a higher percentage of sections from the upper part of the root had two colonies; (2) the percentage of sections with two colonies increased with age from 7 to 87 days. At 87 days in the case of the upper 2 cm. there were appreciably more sections with two colonies than with one colony. The percentage of sections with three colonies never exceeded 6 % and their distribution was not obviously related to the age of the roots.

Colonization behaviour of the most abundant fungi

Two aspects of the colonization of the root surface will be considered under this heading: (1) the rate of colonization of the root surface by particular fungi; and (2) the pattern of root surface fungi on roots of different ages.

Rate of colonization. As above, the upper 2 cm. of root surface was considered separately from the lower 2 cm. Table 4 gives the percentages of root surface (in both groups) colonized by seven fungi for roots of different ages. It is the most commonly occurring fungi that give the most satisfactory picture. The trend of colonization of the less abundant fungi is less readily discerned, since failure to appear on a few sections has a greater effect on the percentages.

Cylindrocarpon. This fungus is the most vigorous and regular colonizer of the surfaces of pea roots. It shows a similar pattern on both the upper and lower portions of the root lengths. By 7 days it was already well established on one-third to one-half of the root surface. The maximum colonization by this fungus was reached at 28 days for the upper part of the root, when three-quarters of the surface was occupied. There was a significant lag on the lower portion, which reached a maximum at 43 days. After this there was a steady decline in abundance on both parts of the root, but more particularly on the upper portion.

Table 4. *Percentage colonization of root surface by most abundant fungi*

Age of roots (days) ...	...	7	14	21	28	37	43	50	87
Cylindrocarpon	U	37	53	40	76	44	46	56	32
	L	45	49	56	48	44	68	62	54
Pythium	U	5	6	7	6	34	16	10	32
	L	2	8	10	6	12	14	8	10
Gliocladium roseum	U	4	7	19	4	6	4	10	64
	L	2	0	2	0	0	0	2	44
Fusarium	U	12	15	38	6	44	50	28	18
	L	15	15	22	18	32	22	26	16
Fusarium, red	U	0·4	3	6	2	2	4	10	6
	L	4	1	2	4	4	2	8	8
Fusarium, white	U	7	7	17	0	0	20	6	4
	L	5	9	10	2	0	12	10	0
Mortierella	U	4	4	4	16	18	0	0	2
	L	3	6	3	22	6	4	2	8

U = upper 2 cm. root surface; L = lower 2 cm. root surface.

Pythium. This fungus was considerably less abundant than *Cylindrocarpon* on both portions of the root. In no case did the amount of root surface colonized by this fungus exceed one-third on the upper portion or one-seventh on the lower portion of the root. Furthermore, this maximum was reached much later than in the case of *Cylindrocarpon.* On the lower portion of the root surface *Pythium* did not greatly increase its hold after the first 14 days. The situation was rather different on the upper root surface where the general trend was towards an increase in the importance of this organism. In particular it is of interest to note that at 87 days *Pythium* had become co-dominant with *Cylindrocarpon.*

Gliocladium roseum. This species is of extreme interest, inasmuch as it is the only fungus found in this investigation that is well able to compete with previously established root surface fungi. It appears to be a late colonizer and did not become particularly abundant until more than 50 days after sowing. At 87 days this organism occurred on over 60 % of the older root sections. It was less abundant on the lower part of the root.

Fusarium. This genus is well represented on the root surface of peas. As with the other fungi, it was more abundant on the upper portion of the root, where it reached a maximum at about 37 days. On the lower portion of the root it reached a maximum at about the same time.

Red Fusarium. This fungus was present to some extent throughout the period that the pea roots were studied, but it never colonized more than 12 % of the root surface.

White Fusarium. Like the red *Fusarium*, this fungus was usually present to some extent on both the upper and lower portions of the root and was similarly slightly more abundant on the upper portion. On both portions it reached its maximum development at 43 days.

Mortierella. This Phycomycete was usually present on the root surface, reaching its maximum development on 28- to 37-day-old roots.

The pattern of fungi on root surfaces of different ages

If the data from this work are analysed with respect to percentage colonization of each 2 mm. root section in each age group by the most abundant fungi, it is possible to obtain a more detailed, if very approximate, picture of the distribution of these organisms along the root length. It appears that, underlying the general trend of colonization of the upper and lower portions of the root surface, there is a continual fluctuation from one age group to the next in the relative abundance of the different fungi. Broadly speaking, if a fungus is particularly abundant on certain sections of the root surface, then other fungi tend to be excluded from these sections. Much remains to be done to clarify the reasons for this apparent jockeying for position on the root surface. It is presumably an expression of such factors as fluctuating environmental conditions, availability of inoculum, competition on the isolation medium and the presence of competing micro-organisms, e.g. soil bacteria, on the root surface. Work is being continued in the hope of defining some of these factors.

Seasonal variation in rate of colonization

Some evidence of a seasonal variation in the rate of colonization of the root surface is given by the data in Table 5. In this the percentage of colonized root surface of roots grown in midsummer–early autumn is compared with that of roots of the same age grown in winter. There is an appreciable decrease in rate of colonization in the winter months.

Table 5. *Seasonal variation in percentage colonization of root surface*

	Age of roots (days)					
	7	14	21	37	43	50
July–Oct. 1956	84	98	100	96	96	95
Dec. 1956–Jan. 1957	25	67	76	98	94	95

Discussion

Many workers have employed washing techniques for the examination of rhizosphere, root-surface and root-infecting fungi (Robertson, 1954; Harley & Waid, 1955). In some cases the difficulties of isolating fungi from a clearly defined substrate have not been fully overcome and the resulting lists contain a mixture of root-surface fungi, root-infecting fungi and general soil fungi. In the present work it soon became clear that simple washing, however prolonged and vigorous, was inadequate to cleanse the root surface sufficiently to justify the conclusion that all the fungi isolated were active on the root surface. Pea roots, particularly if grown in relatively dry soil, have particles of soil firmly stuck to the surface. These can only be removed by a strong jet of water. When this preliminary treatment was omitted, or inefficiently performed, the fungal population contained a number of fungi not usually found on thoroughly washed roots, notably *Mucor*, *Absidia* and *Penicillium*. The efficiency of the present washing technique in removing soil particles and fungal inocula from the root surface was checked by plating-out portions of the wash water, a refinement of technique introduced by Harley & Waid (1955). Towards the end of the series, this was free from fungal inocula. Furthermore, the youngest portions of the roots often remained sterile on the isolation plates. The data presented above indicate that the initial colonization of a virgin root surface takes place very rapidly during the normal growing season. Near the root apex, colonization is patchy and a fungus-bearing section is often sandwiched between sterile sections. On the older root surface there is a rapid lateral spread of fungal mycelium. This tends to be checked when a previously occupied portion of surface is reached. As the root ages, competition for the already occupied portions of the surface becomes more intense and a higher proportion of sections yield more than one fungus on being plated. A point of considerable interest is that almost all the fungi isolated from the root surface of peas have slimy spores.

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FUNGAL MYCELIUM IN DUNE SOILS ESTIMATED BY A MODIFIED IMPRESSION SLIDE TECHNIQUE

By JULIET C. BROWN*

Royal Holloway College, University of London

(With 1 Text-figure)

A modification of the soil-impression slide technique for quantitative purposes, using slides coated with nitrocellulose and a metallurgical objective, is described. By this technique the total amounts of fungal mycelium in an acid and an alkaline dune system were considered in relation to dune succession, soil type and soil profile. Fungal mycelium was found to be more abundant in acid dunes, to increase rapidly with dune development, and in general to decrease down the soil profile, except in the fore dunes where an inversion of the microfungal profile occurred.

INTRODUCTION

A satisfactory method for the quantitative estimate of soil fungi has not been developed. In fact, it is doubtful whether it will ever be possible to determine the 'number' of fungi in a given quantity of soil for, as Burges (1953) has pointed out, the expression 'individual fungus' has no real meaning in the sense that we may talk of individual bacteria. The problem of determining the total weight of fungi in the soil also remains unsolved, since a complete separation of fungal mycelium from the soil, from other micro-organisms, and from its own non-viable component, has not been achieved on a macroscale.

Soil fungal populations have been estimated indirectly from the number of colonies developing in culture (Waksman, 1922), or more directly from measurements of the length, coverage or frequency of mycelial fragments in various soil preparations (King, Hope & Eaton, 1934; Jensen, 1935; Jones & Mollison, 1948). Various cultural techniques, in particular the dilution plate method, have been widely used for determining the size of soil fungal populations. These all have the disadvantage of being over-selective and of reflecting sporing capacity rather than the amount of active mycelium in the soil. Among the direct methods, the Rossi-Cholodny contact slide technique (Cholodny, 1930) has provided useful information on changes in soil microbial populations. The estimates are made, however, on mycelium which has grown over the artificial habitat of the slide, and not on mycelium present in the soil at any given moment. The direct method of Jones & Mollison (1948), in which agar films of constant thickness are prepared from a soil suspension, is ingenious but entails loss of the coarse soil fractions. In the case of young dune soils,

* Now at the Nature Conservancy, Merlewood Research Station, Grange-over-Sands, Lancashire.

where most of the hyphae adhere closely to the sand grains, this has been found to cause the virtual absence of mycelium from the final preparations.

In an investigation of the distribution of soil microfungi in some British dune systems (Brown, 1957), variations in the total amount of mycelium with dune type and dune development have been studied, using a modification of the impression slide technique (Rossi & Riccardo, 1927). The method does not involve removal of the coarser sand grains, and it gives quantitative results on a comparative rather than on an absolute basis.

Rossi, Riccardo, Gesué, Stanganelli & Wang (1936) obtained soil films, or 'impressions', by pressing clean glass slides, or slides smeared with agar or gelatin, momentarily against a prepared soil surface. The microorganisms which adhered to the slides were then fixed and stained. The amount of soil which adheres to a glass slide in this way is too small and variable for quantitative purposes. If, however, a fairly strong adhesive is smeared over the slides, relatively even soil films can be obtained during brief contact with the soil. The adhesive must spread evenly, it must be semi-transparent to allow the passage of light during microscopic examination, and it must not take up fungal stains or mix readily with water. Nitrocellulose (gun cotton), thinned to a convenient consistency with amyl acetate and mixed with castor oil to reduce brittleness, has been found to meet these requirements. The soil films are examined unmounted at high magnifications, without the removal of the coarse mineral particles, by using a metallurgical objective with a long working distance.

THE DUNE SOILS

Soils were examined from the acid dune system at Studland, Dorset, and from the alkaline dune system at Sandwich, Kent. The successive ecological zones sampled in each system included:

Sandwich

(1) The open sand of the tide-washed beach.
(2) Fore dunes.
(3) Semi-fixed 'yellow' dunes.
(4) Semi-fixed to fixed 'grey' dunes.
(5) Old fixed dune pasture.

Studland

(1) The open sand of the tide-washed beach.
(2) Fore dunes.
(3) Semi-fixed dunes, or 'dune grass' (Good, 1935).
(4) Semi-fixed to fixed dune heath.
(5) 'Southern Heath' Callunetum } two facies of dry dune heath developed on the oldest dunes of the system.
(6) *Pteridium–Betula* heath }

METHOD

Five random soil profiles were prepared for sampling in each dune zone. Exposed soil was removed from the vertical face of the profile with a sterile scalpel to prevent contamination of one horizon by another. A soil film was then prepared at depths of 0–1 in. (0–2·5 cm.), 2–3 in. (5·1–7·6 cm.), 5–6 in. (12·7–15·2 cm.) and 11–12 in. (27·9–30·5 cm.) below the surface litter layer, except on the foreshore where, owing to the mobility of the habitat, only surface sand was sampled. Additional films were made in the fore dunes (11–12 in.), dune heath (2–3 in.) and dune pasture (11–12 in.) where the soil horizons were more variable.

The soil films were obtained on sterile glass slides (3 × 1 in.) which had been smeared with nitrocellulose in amyl acetate + 5 % castor oil. The adhesive was spread, by means of a brush, over an area 2 × 1 in. near the centre of the slide, just before the slide was pressed for 20 sec. to the soil. Variations in the adhesive powers of the soil constituents and in the amount of pressure applied may alter the density of the soil films. It is, therefore, important to spread the adhesive thinly and evenly, and to press lightly so that only the material flush with the soil surface adheres. With care, good replicate films can be obtained and they appear to reflect closely the normal spatial arrangements of the soil particles. Excess material not touching the adhesive was removed by gently tapping the slide when the preparation was dry. The soil films were then stained for 1 hr. in phenolic aniline blue (Jones & Mollison, 1948), quickly rinsed in sterile distilled water and finally dried.

The dry mounts were examined by reflected and transmitted light using a combination of an 8 mm. metallurgical objective, bloomed and corrected for uncovered mounts, and a compensating ocular eye-piece (× 25). Presence, or absence, of mycelium was recorded in 200 random microscopic quadrats ($55 \times 55\ \mu$) per slide. Altogether, 1000 quadrats distributed over five replicate slides were examined for each soil type at any one depth. The quadrats were delimited by inserting a ruled glass disk in the eye-piece and the soil films were examined at all depths of focus before a recording was made.

DISCUSSION OF RESULTS

The results are given in Tables 1 and 2, and in Fig. 1. They show the percentage of quadrats in which fungal mycelium was present; this is termed the 'percentage frequency of occurrence of mycelium'. Very fine mycelium believed to be that of actinomycetes is not included. The figures given for the soil organic matter content are average values for the soil profiles.

It is clear from the frequency figures that the mycelial content of the dune soils examined differs widely with dune type, increases rapidly with dune development, and in general decreases with increasing soil depth.

The difference in the mycelial content of the two dune systems is striking. Except in the open sand and fore dunes where soil conditions in the acid and alkaline systems are most alike, the acid dunes contain some

2–4 times as much mycelium as the alkaline dunes. It is widely held that acid soils contain a richer fungus flora than alkaline soils (Waksman, 1924). Warcup (1951) pointed out that this view was based mainly on counts of fungal colonies in culture. Jensen (1931) had concluded, however, from direct microscopical examination that larger amounts of mycelium occurred in acid than in alkaline or nearly neutral soils. The above results support this conclusion. Whether or not soil reaction is directly responsible for this differential distribution of mycelium is uncertain and many factors, including bacterial competition and attack, may be involved.

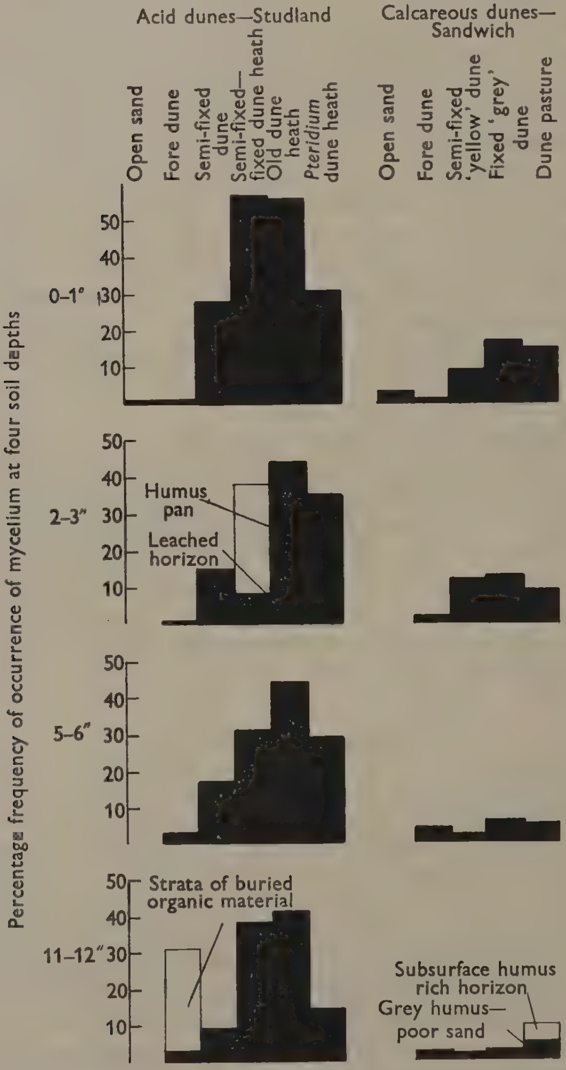

Fig. 1. Percentage frequency of occurrence of fungal mycelium in dune soils estimated by an impression slide technique.

Table 1. *Percentage frequency of occurrence of fungal mycelium in soils of the alkaline dune system at Sandwich*

Dune zone	Soil sampling depth (in.)	Soil profile features	Soil pH range	Soil organic matter content (% loss on ignition)	Percentage frequency of mycelium
Open sand	0–1	—	7·7–9·1	1·3	3
Fore dunes	0–1	Undifferentiated profile	7·4–9·1	0·8	1
	2–3		7·7–9·1	0·7	2
	5–6		7·6–8·9	0·7	4
	11–12		7·5–8·8	0·8	3
Semi-fixed 'yellow' dunes	0–1	Sandy humus horizon covered with wind-blown sand	6·8–9·9	1·5	9
	2–3	Transition to yellow sand	7·4–8·5	1·2	12
	5–6		7·4–8·6	0·7	2
	11–12		7·4–9·2	0·8	2
Fixed 'grey' dunes	0–1	Humus horizon	6·8–7·6	2·2	17
	2–3		7·2–8·0	0·9	13
	5–6	Transition to yellow sand	5·6–8·8	0·8	6
	11–12		7·4–8·3	0·6	3
Dune pasture	0–1	Humus horizon	6·5–7·7	3·2	15
	2–3		7·0–7·7	2·6	9
	5–6	Transition to grey sand	6·9–7·8	2·0	5
	11–12		7·2–8·4	1·0	5
	11–12	Subsurface humus bands	—	4·5	10

Table 2. *Percentage frequency of occurrence of fungal mycelium in soils of the acid dune system at Studland*

Dune zone	Soil sampling depth (in.)	Soil profile features	Soil pH range	Soil organic matter content (% loss on ignition)	Percentage frequency of mycelium
Open sand	0–1		6·4–7·3	0·2	1
Fore dunes	0–1	Undifferentiated profile	6·6–7·0	0·2	1
	2–3		6·8–7·2	0·2	1
	5–6		6·1–7·5	0·2	3
	11–12		6·1–7·5	0·2	3
	11–12	Pockets of buried organic material	—	—	31
Semi-fixed 'dune grass'	0–1	Humus horizon	4·3–5·3	0·7	28
	2–3	Transition to white sand	4·6–5·8	0·2	15
	5–6		4·7–5·8	0·3	17
	11–12		5·4–6·6	0·2	9
Fixed dune heath	0–1	Humus horizon	3·7–4·3	2·0	57
	2–3	Leached horizon	3·6–4·0	0·3	8
	2–3	Indefinite humus pan	3·5–4·0	9·2	38
	5–6	Transition to grey sand with bands of humus	4·0–4·6	0·7	31
	11–12		3·8–4·9	0·3	38
Old fixed dune—'Southern Heath'	0–1	Sandy peat horizon	3·5–4·2	11·9	56
	2–3	Transition to grey sand	3·2–4·0	0·5	44
	5–6		3·5–4·7	0·2	44
	11–12		4·1–4·6	0·2	41
Pteridium dune heath	0–1	Sandy peat horizon with pockets of wind-blown sand	3·1–4·0	2·2	31
	2–3		3·2–4·3	0·5	35
	5–6	Transition to grey sand	3·7–4·6	0·2	29
	11–12		4·0–5·0	0·2	14

The mycelial content of the open shore and fore dunes of both systems is very low, except in the vicinity of pockets of buried organic material. When the dunes become semi-fixed, the amount of mycelium increases rapidly and it reaches a maximum in the surface soils of the fixed dunes. Webley, Eastwood & Gimingham (1952), and Saitô (1955), have found a similar increase with dune succession in the number of fungal colonies developing in culture. Frequencies below the maximum in the *Pteridium* heath at Studland can be related to the presence in this zone of miniature blow-outs and pockets of wind-blown sand around decaying *Pteridium* plants. A slight decrease in the amount of mycelium also occurs in the dune pasture zone of the calcareous system. This may reflect such factors as a decrease in aeration or an increase in the bacterial population. The vegetation cover is more dense, the soil is more compact and bacteria appear, from their growth on Warcup soil plates, to be more prevalent than in the preceding zone of fixed 'grey' dune.

The amount of mycelium at various levels of the soil profile seems to depend both on soil depth and on soil organic content. Thus, although there is a general tendency for the amount of mycelium to decrease with depth from a maximum at the surface, a subsurface increase in organic matter is often paralleled by an increase in mycelial content. In the fore dunes, therefore, the typical microbiological profile tends to be reversed. Here, a humus-rich A_1 horizon is absent, and the upper layers of sand are unstable and often very dry, but at lower depths moisture increases and pockets of organic material occur. In these young profiles mycelial frequency increases slightly with depth, and flushes of mycelium sometimes occur at subsurface levels. Similarly, where the A_1 horizon is covered by wind-blown sand—as on the semi-fixed dunes at Sandwich—the maximum quantity of mycelium occurs just below the surface. Again, in the dune pasture zone a secondary increase occurs where humus bands traverse the subsurface sand. In the podsolized dune heath soils, a marked decrease in mycelial frequency from 57 % at the surface to 8 % in the leached A_2 horizon is followed by an increase to 38 % in the B_1 humus pan, which confirms similar reports on the nature of the microbiological profiles of podsols (Gray & Taylor, 1935; Jefferys, Brian, Hemming & Lowe, 1953).

The increase in frequency of mycelium in subsurface humus horizons is not as great as might be expected from the organic content alone. This supports earlier observations by Timonin (1935) and Stenton (1953) who found that micro-organisms decreased with increasing soil depth, despite high concentrations of organic matter.

In general, numbers of colonies on Warcup soil plates have reflected variations in the fungus population found by the impression slide technique. Rather more mycelium occurred, however, in the soil films from lower levels of the acid dunes than was to be expected from colony numbers, possibly due to a decrease in sporing capacity with depth. Moreover, mycelium was sometimes frequent in soils which appeared to be practically sterile when cultured, indicating incomplete isolation of the soil fungal population by the soil plate method.

Satisfactory films of the peaty horizons were sometimes difficult to obtain because of clumping of the sticky soil aggregates. The frequency figures obtained for these organic layers may, therefore, be somewhat low. Some error may also have resulted from the difficulty of distinguishing between viable and non-viable dematiaceous hyphae by the usual staining techniques. A further disadvantage, shared by other direct observational methods, is that the mycelium cannot usually be identified on vegetative characters alone. Features such as septation, branching and colouring were, however, often clearly visible and sporophores were occasionally seen.

The merits of this modified impression slide technique are that it is quick and simple to apply; it enables comparisons between different soil types to be made on a soil volume instead of a soil weight basis and, unlike Rossi-Cholodny slides, it indicates the *status quo* of the total mycelial content without undue disturbance of the soil. The technique is designed primarily for the examination of soils which are low in organic matter, and has proved of special value in coarse grained sands of the dune type.

I am most grateful to Prof. F. W. Jane and Dr M. P. Topping who supervised this research, which was carried out during the tenure of a Nature Conservancy Studentship.

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STUDIES ON *PUCCINIA POLYSORA* UNDERW.

I. THE WORLD DISTRIBUTION OF FORMS OF *P. POLYSORA*

By R. H. CAMMACK

West African Maize Research Unit, Ibadan, Nigeria

(With 2 Text-figures)

An examination of herbarium material of *Puccinia polysora* from all areas of the world revealed two main size groups of uredospores. The smaller group was encountered in South East Asia and neighbouring islands with the exception of Borneo, and the larger group in the West Indies, Africa and the South Indian Ocean. The two main groups are consistent with the two directional spread, westwards and eastwards, from the origin of the rust, the Caribbean area.

As data on cross-inoculation studies are lacking it is uncertain if distinct forms of *P. polysora* exist.

Until the appearance of maize rust caused by *Puccinia polysora* Underw. in West Africa in 1940, knowledge of the world distribution of this rust was confined to reports from Central and South America and the West Indies. The first record was established by Underwood (1897), who described it as new from *Tripsacum laxum* in Alabama. Arthur (1920) recorded it on *T. laxum* in Florida, New Jersey and San Domingo and on other species on *Tripsacum* in Mexico and Cuba. Cummins (1941) identified it on *Zea mays* from Peru and later found it to be of general occurrence on maize in Central and South America. Other records are given by Wood & Lipscomb (1956).

THE WORLD SPREAD OF *PUCCINIA POLYSORA* ON *ZEA MAYS*

Central and South America, West Indies

In 1945 the rust was observed on *Zea mays* in Trinidad and in 1947 in British Honduras. In 1948, it was recorded in Jamaica. Information concerning the severity of the disease in these areas is almost lacking, but it was observed that 'negligible' damage was caused in the West Indies.

South-East Asia and adjacent islands

Specimens of maize rust were collected in Malaya in 1950 and identified as *P. polysora*. In 1952 maize in British North Borneo was affected and in 1956 Siam, the Philippines and Christmas Island (Indian Ocean). Again reports of damage are scanty but, in some of the affected areas the attack was widespread and locally severe.

The African Continent

Deighton (1951) reported that maize rust during the 1949 growing season was widespread and severe in Sierra Leone. The rust was at first thought to be *P. sorghi*, previously only once recorded in Sierra Leone (in 1943), but the virulence of the disease aroused suspicions, and a critical examination was made by G. R. Bisby, of Sierra Leone specimens collected in 1949 and of collections made in 1951 from Ghana and Nigeria, as a result of which he concluded that they belonged to *P. polysora*, and G. B. Cummins confirmed these identifications. After further examinations of all maize rust specimens from the African Continent, it was determined with reasonable certainty that *P. polysora* did not occur in Africa before 1949.

In 1950 the Department of Agriculture, Ghana, reported a generally distributed outbreak of maize rust in that colony, and it was observed that the highest incidence of rust was in the coastal rain-forest areas, where rainfall and humidity are greatest and was less severe in the drier areas of the interior. All specimens collected in Ghana during 1950 were identified as *P. polysora*. In mid-1950 the Department of Agriculture of the French Territories of Ivory Coast and Dahomey reported outbreaks of rust, and mentioned that rust was most severe on maize planted late in the season

Table 1. *The world distribution of* Puccinia polysora

(Dates of first appearance are listed in chronological order.)

Territory	Year first reported	C.M.I. Herbarium no.	Host
Alabama	1897	Det. Underwood	*Tripsacum laxum*
Florida	1920	Det. Arthur	*T. laxum* *T. latifolium*
Peru	1941	Det. Cummins	*Zea mays*
Trinidad	1945	1944	*Z. mays*
Honduras	1947	47428	*Z. mays*
Jamaica	1948	31440	*Z. mays*
Sierra Leone	1949	38678	*Z. mays*
Ivory Coast	1950	48685	*Z. mays*
Ghana	1950	45931	*Z. mays*
Dahomey	1950	47944	*Z. mays*
Nigeria	1950	45926	*Z. mays*
Malaya	1950	62164	*Z. mays*
French Guinea	1951	53057	*Z. mays*
Belgian Congo	1952	52565	*Z. mays*
Sudan	1952	51198	*Z. mays*
Kenya	1952	50121	*Z. mays*
Tanganyika	1952	50581	*Z. mays*
North Borneo	1952	62556	*Z. mays*
Nyasaland	1953	52590	*Z. mays*
Northern Rhodesia	1953	52489	*Z. mays*
Southern Rhodesia	1953	56016	*Z. mays*
Portuguese S. Africa	1953	Det. Carvalho	*Z. mays*
Madagascar	1953	—	*Z. mays*
Mauritius	1953	51927	*Z. mays*
Reunion	1953	52909	*Z. mays*
Agalega Island	1955	60590	*Z. mays*
Rodriguez Island	1955	60203	*Z. mays*
Christmas Island (Indian Ocean)	1956	62093	*Z. mays*
Siam	1956	Det. Cummins	*Z. mays*
Philippines	1956	Det. Cummins	*Z. mays*

and that early plantings escaped to some extent. In late 1950 the rust appeared in the south-west corner of Nigeria (Rhind, Waterston & Deighton, 1952). The following year the rust was widespread throughout western and eastern Nigeria and had appeared in Cameroons under British Trusteeship. Nattrass (1952) reported *P. polysora* in Kenya in 1952. The previous year, having been warned of the advent of *P. polysora* in West Africa, an examination of maize rust in the colony had revealed only *P. sorghi*. Also in 1952 it was reported in the Belgian Congo, Tanganyika and the Sudan. Early in 1953 the rust appeared in Nyasaland and Southern Rhodesia, and later the same year in Madagascar, Mauritius and Reunion in the South Indian Ocean. In 1955 further reports were received from the Islands of Agalega and Rodriguez in the Indian Ocean.

The present known world distribution of *P. polysora* is given in Table 1 (see also C.M.I. distribution map 237) and when there is a specimen at the Commonwealth Mycological Institute a herbarium reference number is given.

VARIATION IN UREDOSPORE SIZE

In order to determine if uniformity existed in the dimensions of uredospores an analysis was made of collections from different areas in West Africa. Samples of a hundred spores from herbarium specimens from each of the following locations were measured:

Territory	No. of samples	Territory	No. of samples
Sierra Leone	3	Nigeria	14
Ivory Coast	2	Cameroons under British Trusteeship	3
Ghana	11		
Dahomey	2		

Frequency distribution curves were constructed from 500 measurements of lengths and breadths of uredospores collected at Ibadan, Nigeria, during 1953, and all other West African samples listed above were subsequently compared with these curves, and it was found that none departed significantly from the size distribution of the Ibadan sample. An analysis of uredospore measurements of West African specimens is given in Table 2.

In 1956 F. C. Deighton sent the writer specimens of maize rust from South East Asia and adjacent islands, and mentioned that uredospores from that area were apparently smaller than in collections from Africa. Six samples, each of 100 spores, were measured from Malayan material,

Table 2. *An analysis of the measurements of uredospores of* P. polysora *from collections made in West Africa and the Eastern Pacific*

	West Africa	Malaya, Christmas Is.
Mean length (μ)	32·70 ± 0·14	28·67 ± 0·23
Mean breadth (μ)	24·79 ± 0·24	22·59 ± 0·31
Correlation coefficient of length to breadth	−0·28	−0·31
Relative volume of spores (Smith, 1953)	1·0	0·78

Mean difference of lengths (μ) 4·03 ± 0·38
Mean difference of breadths (μ) 2·20 ± 0·24

and two from Christmas Island (Indian Ocean). The analysis of these samples is also given in Table 2, and the results show a highly significant difference ($P=0{\cdot}1\ \%$) between West African, and Malayan and Christmas Islands (Indian Ocean) spores both in lengths and breadths.

With the assistance of the Commonwealth Mycological Institute and the Arthur Herbarium, duplicates of all available specimens of *P. polysora* on *Zea mays* and on the alternate hosts *Tripsacum laxum* and *Euchlaena mexicana* were obtained. Samples of 100 spores from a total of sixty-five specimens, representative of all areas of the world affected by the rust, were measured.

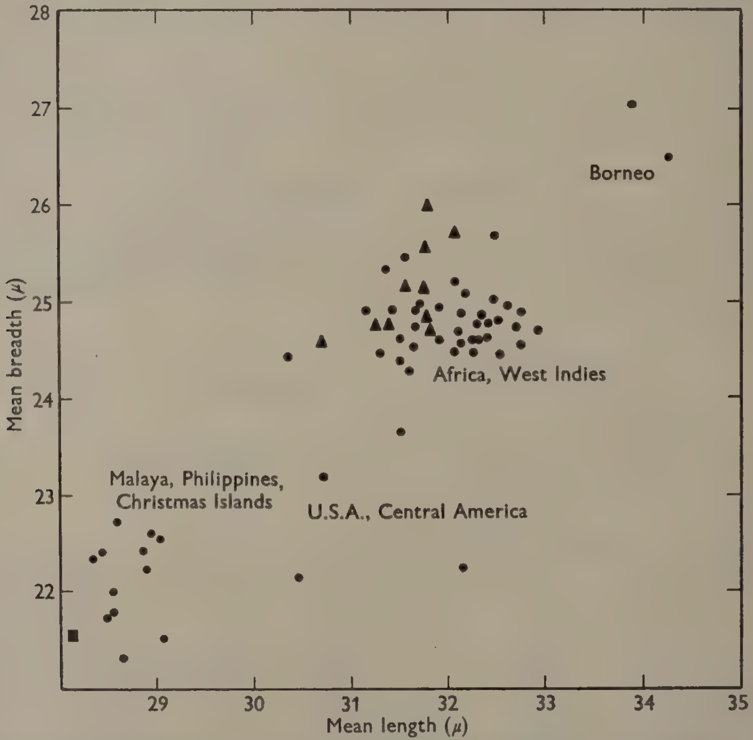

Fig. 1. The distribution of mean measurements of uredospores of *Puccinia polysora*. Host key: ●, *Zea mays*; ▲, *Tripsacum laxum*; ■, *Euchlaena mexicana*.

Uredospores from herbarium specimens which have been identified as *P. polysora* fall on a size basis into two main groups. This grouping is illustrated in Fig. 1 in which the mean size of samples is plotted. Samples obtained from different hosts are indicated by different symbols. This figure shows that there are two main forms of *P. polysora* to be found on *Z. mays* when distinguished on a size basis. The smaller of these is centred on the Pacific area and is consistent with the eastward spread of the rust from its first recorded appearance in South-East Asia, i.e. from Malaya. Presumably the origin of the rust was once more America. Records from Borneo, however, are not consistent with this, the spores being much larger (Figs. 1, 2). The larger spore form is found in the Caribbean area, Africa and islands in the South Indian Ocean and is consistent with the observed eastward spread (Table 1).

Fig. 2 illustrates the variation in morphology with location. The histograms were each constructed from 200 measurements from samples of rust chosen at random from the areas mentioned. The mean size of each sample is marked by a vertical line.

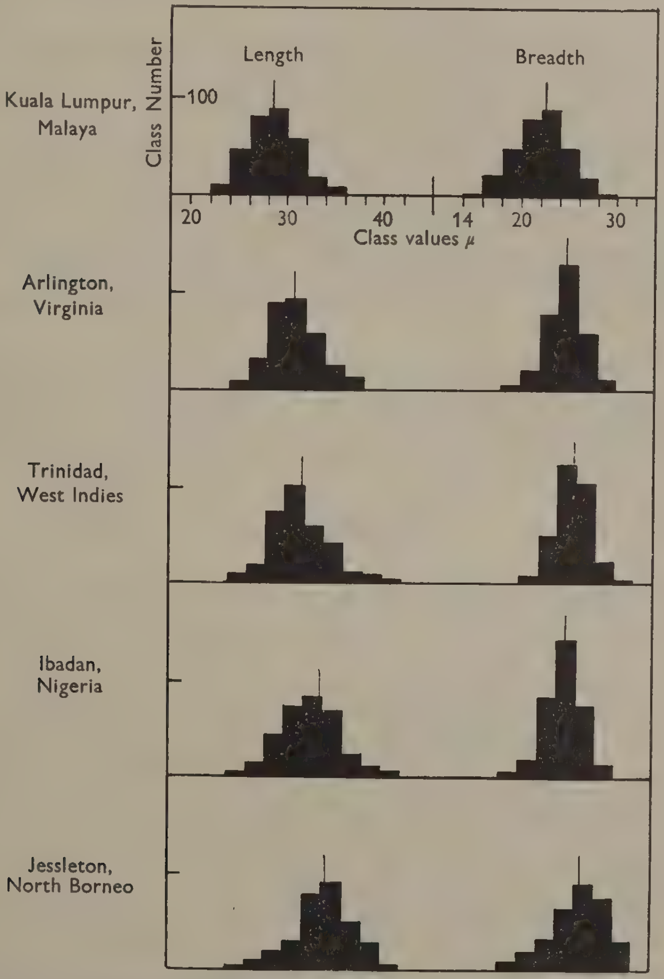

Fig. 2. Class-frequency histograms of the measurements of five samples of uredospores of *Puccinia polysora*.

Discussion

Data are lacking on cross-inoculation studies of cultures of *Puccinia polysora* from areas outside Africa and it is not possible at present to determine if biotypes exist. Attempts to infect *Tripsacum laxum* at Ibadan with West

African uredospore material obtained from *Zea mays* have failed. The *Tripsacum* used was clonal material from the West Indies in which area natural infection of this host by *P. polysora* occurs readily.

However, it has not been established whether the forms of *P. polysora* on *Zea* and *Tripsacum* in the West Indies are identical. Further investigations are required to determine this point, using cross-inoculation and host material of known genetic identity. Until this has been done there is no justification for further assumptions on the relationship of the groupings in Fig. 1.

The effect of environment on uredospore formation may be a contributory factor to the variations in spore size that have been found. Levine (1923) and Stakman & Levine (1919) have shown that environmental factors influence uredospore size in *P. graminis*, and Bailey (1925) observed that environmental conditions result in changes in uredospore size as great as, or even greater than, the differences between spores of different races of *P. g. avenae* produced under similar conditions. Hingorani (1952) showed that significant fluctuations were induced in uredospore size of races 2, 7 and 8 of *P. g. avenae* by temperature, light intensity and the degree of resistance of the host. This last factor, especially, may be applicable to *P. polysora*.

Levine (1923) and Waterhouse (1930), and Manners (1950) have shown uredospore size variation in different physiologic races of *P. graminis* and *P. glumarum*, respectively. A pleiotropic effect of this type is not unexpected in *P. polysora* on theoretical grounds.

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PORIA HEALEYI SP.NOV. THE CAUSAL FUNGUS OF YELLOW STRAW ROT IN JARRAH (*EUCALYPTUS MARGINATA* SM.)

By N. E. M. WALTERS

Division of Forest Products, C.S.I.R.O., Australia

(With Plate 1 and 2 Text-figures)

A description is given of *Poria healeyi* which causes a decay (yellow straw rot) in the Western Australian timber, jarrah (*Eucalyptus marginata* Sm.), but which fruits readily only in eastern Victoria where its principal host is *E. regnans* F. Muell.

Type material has been deposited in the herbaria of the Division of Forest Products, C.S.I.R.O., Australia; of the Royal Botanic Gardens, Kew, England; and of Dr G. H. Cunningham, Auckland, New Zealand.

Jarrah (*Eucalyptus marginata* Sm.) is one of the most important commercial timbers in Australia. It occurs only in a restricted zone in south-western Australia, where it is reasonably abundant. It is very durable by oversea standards and hence relatively few fungi attack it to a serious degree. For many years, however, foresters of that locality have been familiar with a rapid decay of fallen jarrah logs known there as 'yellow straw rot', but no associated fruit-body has ever been reported, even after sustained search.

About twenty years ago, the decays of this timber (among others) were extensively investigated by Tamblyn (1937) who repeatedly isolated the causal fungus of yellow straw rot. Its culture on malt agar was very pale off-white and thin at first but in a few days, on a definite, even advancing front, changed to a warm apricot felt and in a short while malformed pores developed. The causal fungus was therefore believed to be a polypore of some kind, possibly resupinate.

In 1954 the author received from Mrs K. Healey a specimen of a *Poria*, which was submitted to Dr G. H. Cunningham who considered it identical with an unnamed *Poria* sent him by Dr J. B. Cleland of South Australia some time before. The tissues of both were choked with a gum or resin and were accordingly unidentifiable. Mrs Healey's specimen was collected on a fallen log of mountain ash (*E. regnans* F. Muell.) another important but less durable timber, in Tarra Valley National Park, eastern Victoria. Later, by request, she sent a number of other specimens from the same locality.

When the culture was isolated, the characteristic appearance of Tamblyn's Western Australian fungus was immediately observed in it. It too produced pores and a light white spore cast. Both the cultures were induced to produce perfect fruit-bodies in the laboratory and it was then clearly established that the two fungi were identical.

The hymenial structure of the fungus was revealed by dropping hand-cut sections from a young fruit-body into acetone, which extracted the red 'gum' in a few minutes. The acetone was then removed gently by adding water slowly and allowing the overflow to drip away at the same rate. A clear hyaline section resulted. This could not be done with older specimens, which would not even discolour the acetone. Even so, the connexion between the old and new forms has been established beyond doubt in the field and can also be seen in the laboratory when a large young specimen dries out.

Poria healeyi sp.nov. (Pl. 1, Text-fig. 1)

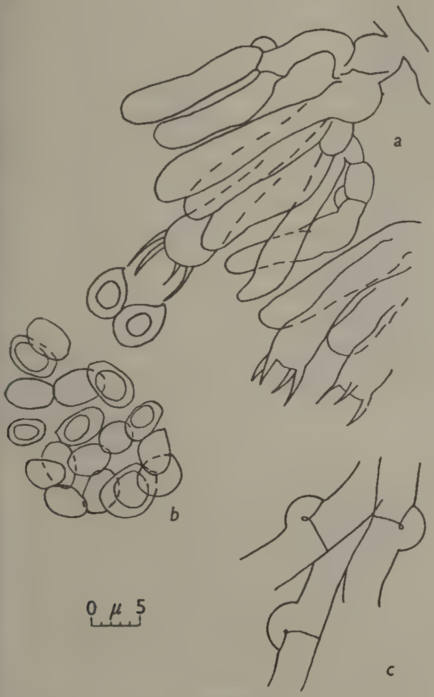
Hymenophorum ad 30 × 20 cm., ad 2 cm. crassum, molle vel coriaceum, roseo-ochraceum, gummiferum, siccitate corneum et resiniferum, castaneo-nigrum. Pori rotundati, ad 2 cm. profundi, 150–200 μ diam.; dissepimenta 75–100 μ crassa. Contextus roseo-ochraceus, siccitate castaneo-niger, ad 1 mm. crassus, textilis. Hypharum systema monomiticum. Hyphae generatoriae 4 μ crassae, ramificatae septatae, fibulatae. Typus basidialis clavatus. Sporae ovatae vel ellipticae, 3·5–5·5 × 3·0–4·5 μ, leves, hyalinae. Specimen typus (pars) in Herbario Regale Kewense depositus est.

Fruit-body resupinate (perennial in a few specimens but not in the type), in extensive patches to 30 × 20 cm. and to 2 cm. thick, soft, velutinate, apricot to orange in youth (Apricot Orange to Ferruginous—Ridgway XIV), bruising to red, becoming lustrous chestnut-black, curling up and cracking in age and then often with sharply contrasting young thin growth covering its pored surface. *Pores and tissues* saturated from an early age with a reddish orange gummy substance, which, after a few months, or on drying out, becomes darker and resinous in appearance. *Hymenium* without cystidia. *Basidia* 4 μ broad, projecting to 8 μ, clavate type (sensu Cunningham, 1947) with four sterigmata 4–6 μ long (Text-figs. 1, 2). *Hyphal system* monomitic. Hyphae closely woven, thin walled, 4 μ in thickness, hyaline, with clamp connexions. Dissepiments 75–100 μ thick, narrower than the tubes, which are 150–200 μ diam. and 4 to 1 mm. *Spores* smooth, hyaline, staining pale blue in cotton blue, elliptical to ovoid, 3·5–5·5 × 3·0–4·5 μ, often with a guttule (Text-fig. 1).

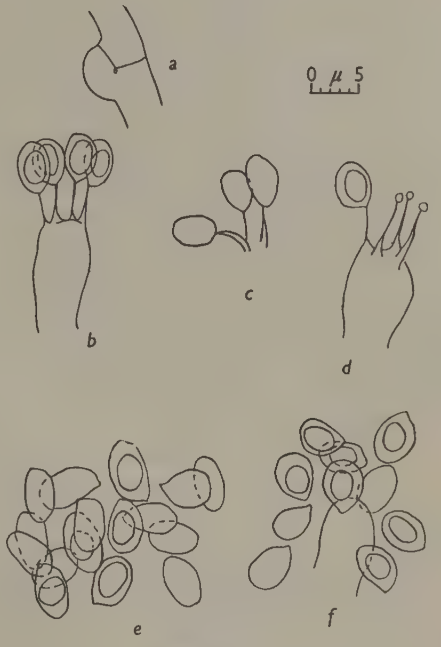
Cunningham (private communication) believes the fungus is allied to *Poria mollusca* (Pers. ex Fr.) Cke. It appears to differ substantially from this species, however, in its gummy secretion, the greater thickness of its fruit-body, its colour and in spore shape. As no description could be found, it is here described as a new species.

The general appearance of the culture has already been noticed. Its hyphae possess clamps as in the fruit-body and the culture has the faintly acid odour of swamps when it is actively decaying wood. The decay produced is a white rot and varies a little in appearance with the host from a yellow mottling or stringy rot in jarrah to a white pocket or stringy rot in its Eastern hosts. It is a column heart rotting fungus which can continue its attack after the tree is dead, at least in certain species.

Eastern Australian specimens have nearly always been found on fallen trunks of *Eucalyptus regnans* F. Muell. but in addition, once on *Acacia dealbata* Link and once on *A. melanoxylon* R. Br. It had in one instance grown from its original host of *Eucalyptus regnans* on to a tree fern trunk lying beneath it. It may be mentioned here that laboratory tests show that the fungus is also able to decay *Pinus radiata* D. Don and *Nothofagus cunninghamii* Oerst. Tamblyn (1937) reports some probable alternative hosts in Western Australia besides jarrah, viz. *Eucalyptus patens* Benth., *E. staerii* Maiden, *E. jacksoni* Maiden, and *E. guilfoylei* Maiden.



Text-fig. 1



Text-fig. 2

Text-fig. 1. *Poria healeyi* sp.nov. Camera lucida drawings of D.F.P. 4246 (type specimen). *a*, portion of hymenium; *b*, spores (hyaline, staining blue in cotton blue); *c*, generative hyphae from the trama.

Text-fig. 2. *Poria healeyi* sp.nov. Camera lucida drawings of D.F.P. 1274 (*ex* W. Australia). *a*, clamp connexion from culture; *b*, *c*, *d*, portions of basidia with spores in various stages of development; *e*, spores from white spore cast; *f*, spores from section of hymenium.

Type material has been sent to Dr G. H. Cunningham, and more is being deposited with the Royal Botanic Gardens Herbarium at Kew where a portion of a perennial specimen (4669) is already located (Pl. 1, fig. 4). Material held in this collection includes the type (D.F.P. 4246, Pl. 1, fig. 3, and Text-fig. 1) and, also from the type locality, Tarra Valley Park, 4026, 4669 (Pl. 1, fig. 4), 4897, 5469, 5470, 5525, 5584 (Plate 1, fig. 1), 5601, 5615, 5621, 5682, 5683, 5690, 5691, 5782, 5783 and 6331 (Plate 1, fig. 2) to 6335 inclusive. In addition, the following Western Australian decay isolates have produced the fruit-body in this laboratory: 1273, 1274 (Text-fig. 2) (leg. N. Tamblyn, October 1947 on jarrah at Manjimup) and 5819 (leg. N. Tamblyn, December 1955 on jarrah at Palgarup).

DISCUSSION

Tarra Valley National Park can be described as an area of virgin temperate rain forest, and the sites that encourage the fungus are usually hemmed in by ferns and near to running water, with very little light filtering through. The contrast between the conditions of Tarra Valley (50 in. rainfall and low light intensity*) and those of the Western Australian jarrah forests (20–50 in. rainfall but higher light intensity) may explain why the fruit-bodies have never been found in the latter.

It is rather remarkable that an environment which strongly favours the vegetative activity of a fungus does not allow it to fruit. It is more remarkable that in the apparent absence of fruiting in Western Australia there is a wide dissemination to the point of abundance. There is only conjecture so far to explain this. For instance, there may be small pockets of territory with micro-climate, suitable to fruiting in or near to the jarrah forests, or the fruit-bodies may appear in abundance in some years and never between. The eastern states cannot be an infection reservoir for the west because some thousand miles of desert or semi-desert lie between. However, Tamblyn (verbal) suggests a possibility that has not yet been investigated. He has noted that the yellow straw rot (among others) frequently follows the course of pinhole borer tunnels. These beetles are adult (imago) insects that usually bore radially inwards from a scar in the living tree and can continue throughout the trunk. The micro-climate in the bore holes may allow fertile basidia to form there, or the emerging beetles may transmit infection by carrying small inocula of living hyphae.

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EXPLANATION OF PLATE 1

Poria healeyi sp.nov.

Fig. 1. Fresh, vigorously growing fruit-body *in situ* (D.F.P. 5584), together with a diffuse white resupinate species of the Hydnaceae. Note the ferny habitat.

Fig. 2. Fresh, vigorously growing fruit-body (D.F.P. 6331) to show its growing margin and pores. × 7.

Fig. 3. Type specimen (D.F.P. 4246). Portions of fruit-body showing resinous lustre of tubes.

Fig. 4. Old cracked fruit-body (D.F.P. 4669) with young growth coating the interstices.

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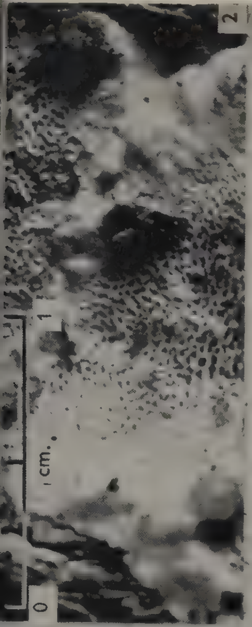
* Rainfall records for Tarra Valley are too recent to be considered accurate. Local opinion suggests that a high humidity, equivalent to a higher rainfall, is a strong factor in the micro-climate there.



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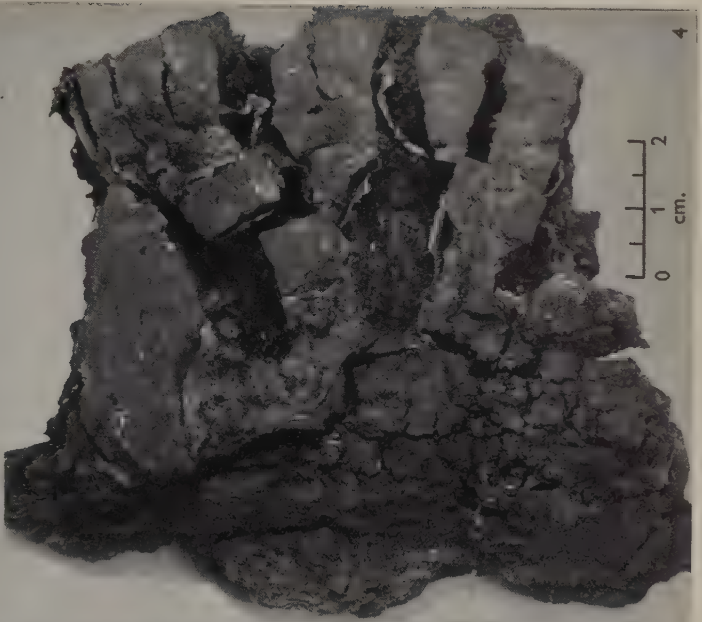

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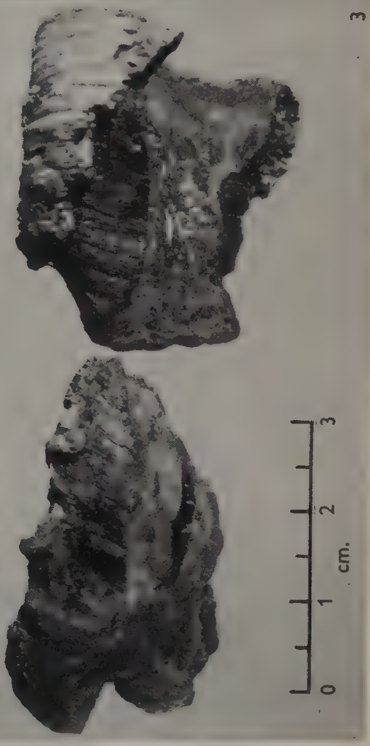

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Trans. Brit. mycol. Soc. **41** (1), 99–108 (1958).

PHYSIOLOGIC SPECIALIZATION IN *RHYNCHOSPORIUM SECALIS*

By HAROLD OWEN

Department of Agricultural Botany, University of Reading

(With Plates 2 and 3 and 4 Text-figures)

British isolates of *Rhynchosporium secalis* show strict host specialization. Five isolates from Argentina and one from Australia behave similarly. Differences in pathogenicity are not apparently correlated with morphological characters of the spores, nor, except to a limited degree, with cultural features.

INTRODUCTION

Rhynchosporium secalis (Oudem.) J. J. Davis, a hyphomycete, causes a disease of barley, rye and various grasses, known as 'leaf blotch' in Britain, and 'scald' in North America (Anon., 1945; Caldwell, 1937). *R. orthosporum* was described by Caldwell (1937) as causing leaf blotch of *Dactylis glomerata* L. in U.S.A., and has since been recorded on *D. glomerata* in England by the writer (Owen, 1952). The present paper deals only with *R. secalis*.

Caldwell (1937) in U.S.A. found a high degree of specialization within *R. secalis*. Six races were described, each restricted to one of the following host plants: barley, rye, *Agropyron repens* (L.) Beauv., *Bromus inermis* Leyss., *Elymus canadensis* L. and *Hordeum jubatum* L. Each race was stated to show constant and distinctive features in culture.

Sarasola & Campi (1947) reported that *R. secalis* isolated from barley in Argentina was capable of attacking not only this host but also *E. canadensis*, *A. smithii* Rudb., *Festuca elatior* L. and *B. catharticus* Vahl.

When the present investigation began, previous workers in Europe had stated or implied that *R. secalis* does not show physiologic specialization. Brooks (1928) recorded the fungus for England on barley, rye, *B. mollis* L., *B. sterilis* L. and *D. glomerata* (probably the fungus on the last was *R. orthosporum*). Apparently he performed no cross-inoculation experiments, but stated '. . . *R. secalis* occurs on a considerable number of wild grasses, and these undoubtedly may be a source of infection to crop plants'. Smith (1937) reported infection of *Agropyron* spp. in England with spores of *R. secalis* from barley, and vice versa. Bartels (1928) concluded from cross-inoculation experiments in Germany that *R. secalis* was not divided into physiologic races, but was a plurivorous parasite attacking the following cereals and grasses: barley, rye, all species of *Poa*, *Bromus* and *Lolium* which were tested, *Hordeum murinum* L., *Agrostis stolonifera* L., *Cynosurus cristatus* L., *Agropyron repens*, *Phleum pratense* L. and *Holcus lanatus* L. Viennot-Bourgin (1949) supported this view and stated that in France he found it easy to infect *B. mollis*, *Hordeum murinum* and *H. secalinum* Schreb. with *R. secalis* isolated from barley.

Müller (1953), in Zürich, made cross-inoculations with *R. secalis* from barley and rye, and found that each attacked only the host from which it was derived. This is the first published statement from Europe that *R. secalis* shows physiologic specialization.

In the present work observations have been made on British isolates of *R. secalis* from several hosts, and also a few isolates from overseas.

MATERIAL

Rhynchosporium secalis has been collected on barley, rye, *Hordeum murinum* and *Agropyron repens*; symptoms on each host are illustrated in Pl. 2.

The isolates used are listed in Table 1. All cultures made by the writer from lesions on leaves were derived from single spores. Isolates were maintained on potato-carrot agar slopes.

Table 1. *Sources of material*

(CBS = Centraalbureau voor Schimmelcultures)

Isolate no.	Host	Source	Culture received from
765	Barley	Sonning, Berks, England	—
772	Barley	Shinfield, Berks, England	—
780	Barley	Germany	CBS (ex Bartels)
781	Barley	Cambridge, England	CBS (ex Brooks)
782	Barley	Argentina	CBS
802	*Hordeum murinum*	Reading, Berks, England	—
806	*Agropyron repens*	Reading, Berks, England	—
808	*A. repens*	Reading, Berks, England	—
814	*Hordeum murinum*	Reading, Berks, England	—
831	Barley	Bedford, England	—
837	*H. murinum*	Reading, Berks, England	—
853	*H. leporinum*	Australia	Prof. W. L. Waterhouse
854	Barley	Bridgham, Norfolk, England	—
856	Barley	Eye, Suffolk, England	—
860	Barley	Norfolk, England	—
864	Barley	Tillingham, Essex, England	—
872	Barley	Stow Bridge, Norfolk, England	—
880	Barley	Harpenden, Herts, England	—
884	Barley	Australia	Miss D. E. Shaw
885	Barley	Guatraché, Argentina	Dr J. A. Sarasola
886	Barley	Guatraché, Argentina	Dr J. A. Sarasola
887	*H. leporinum*	La Vinícola, Argentina	Dr J. A. Sarasola
888	Rye	Castelar, Argentina	Dr J. A. Sarasola
889	Barley	Castelar, Argentina	Dr J. A. Sarasola
907	Barley	Cirencester, Glos, England	—
911	Barley	Warsash, Hants, England	—
919	*Agropyron repens*	Sonning, Berks, England	—
921	Barley	Sonning, Berks, England	—
924	Barley	Winchester, Hants, England	—
930	*A. repens*	Shinfield, Berks, England	—
933	*Hordeum murinum*	Shinfield, Berks, England	—
937	Barley	Goring, Oxon., England	—
941	Barley	Faringdon, Berks, England	—
942	Rye	Thursley, Surrey, England	—
946	*Agropyron repens*	Thursley, Surrey, England	—
954	Barley	White Horse Hill, Berks, England	—

INOCULATION EXPERIMENTS

Methods

Inoculum was obtained from cultures, where conidia are produced more abundantly than on host plants. Caldwell (1937) used mainly conidia from culture in his experiments, but sometimes used host-borne conidia. Bartels (1928) used host-borne spores almost exclusively. In neither instance did the source of inoculum affect the results of the experiments.

In a few early experiments conidia were obtained from slope cultures, but generally the technique described by Sarasola & Campi (1947) was used. Each culture was grown on 50 ml. of potato-dextrose agar in a 250 ml. conical flask, and 5 ml. of sterile distilled water was added at the time of seeding. The fungus spread relatively quickly and formed a complete mat in 8–14 days. The mat was peeled off and macerated for 1 min. with 100 ml. of sterile distilled water, the suspension then being strained through muslin to remove large mycelial fragments.

Each species of Gramineae tested was represented by six plants in a 4 in. pot. The cultivars of barley and rye used were Spratt-Archer and Star, respectively. The pots stood above water in a metal tray, and when the spore suspension had been atomized on to the plants, they were covered with a Perspex chamber and maintained at 20° C. After 48 hr. the plants were removed to a greenhouse at approximately 20° C., where symptoms appeared after 8–12 days. Confirmation that infection had occurred was obtained by examining portions of lesions microscopically.

Results

Rhynchosporium secalis *from barley*

Isolates 765, 772, 781, 782, 831, 854, 856, 860, 864, 872, 880, 884, 885, 886, 889, 907, 911, 921, 924, 937, 941 and 954 were inoculated on the following species: barley, rye, *Agropyron repens*, *Hordeum murinum*, *Dactylis glomerata*, *Bromus mollis*, *B. sterilis*, *Lolium perenne* L. and *Phleum pratense*. Isolates 765 and 781 caused no infection; all others produced infection of barley but of no other species.

Rhynchosporium secalis *from rye*

Isolates 888 and 942 were inoculated on the same species as the barley isolates, and caused infection of rye only.

Rhynchosporium secalis *from* Agropyron repens

Isolates 806, 808, 919, 930 and 946 were inoculated on the same species as the barley isolates, in some cases several times, without infection being produced on any plant.

Rhynchosporium secalis *from* Hordeum murinum

Isolates 802, 814, 837 and 933 were inoculated on the same species as the barley isolates and in each case caused infection of both barley and *H. murinum*.

Four isolates from barley (772, 860, 886 and 889) were tested also on barley, wheat (cv. Squareheads Master), oats (cv. White Winter), *Agrostis stolonifera*, *Bromus catharticus*, *Cynosurus cristatus*, *Festuca elatior*, *Holcus lanatus* and *Poa pratensis*. Only barley became infected.

Two isolates from *Hordeum murinum* (802, 837) and two isolates from *H. leporinum* (853, 887) were tested also on barley, *H. jubatum*, *H. leporinum*, *H. murinum* and *H. secalinum*. Both isolates from *H. murinum* infected only *H. murinum* and barley. Isolate 853 from *H. leporinum* caused no infection; isolate 887 infected *H. leporinum* and *H. murinum*.

SPORE MORPHOLOGY

Methods

The aim was to examine a spore sample from leaves and a sample from culture of each isolate in the collection. This plan could not be fulfilled completely since no spores from leaves were available in the case of a few isolates, and one isolate did not form spores in culture. Each sample consisted of 50 spores taken at random.

Leaf-borne conidia were obtained from inoculated plants. Leaves bearing lesions were incubated in a damp chamber at 20° C. for a day or so to stimulate sporulation. Cultures for the production of conidia for examination were grown on potato-carrot agar slopes incubated at 20° C. Spores were removed after 7–10 days.

All spores were mounted in water, and drawn at constant magnification × 2000 with the aid of a camera lucida. Measurements were taken from the drawings.

Results

Spore shape. Some differences between spores from leaves and spores from culture were noted, especially in certain isolates. In general, however, each isolate produced spores having the same shape in culture and on the plant: spores from both sources had approximately the same length/width ratio, prominence of beak, and degree of over-all curvature. There was much variation in spore shape among the isolates from each host. It did not appear that the isolates from any host showed features of spore shape in common which distinguished them as a group from the isolates from other hosts. Some representative spore samples are shown in Text-figs. 1–4.

Spore size. Both length and width of spores were measured, and variation in them analysed. To conserve space only length will be dealt with here.

Table 2 shows the form of analysis adopted for the initial study of spore length in the twenty-six isolates for which spores were available both from leaves and cultures. The ratio Error (1)/Error (2) is highly significant, and Error (1) is therefore used as the error variance against which source and host variances and their interactions are tested. Spores from leaves are significantly longer than spores from cultures. The interaction Sources × Isolates may be tested against Error (2), and is highly significant, so that Error (2) cannot be used to compare differences between isolates. Hence separate analyses for spores from leaves and spores from cultures were undertaken (Tables 3, 4). It is thus possible to effect a more precise comparison between isolate means.

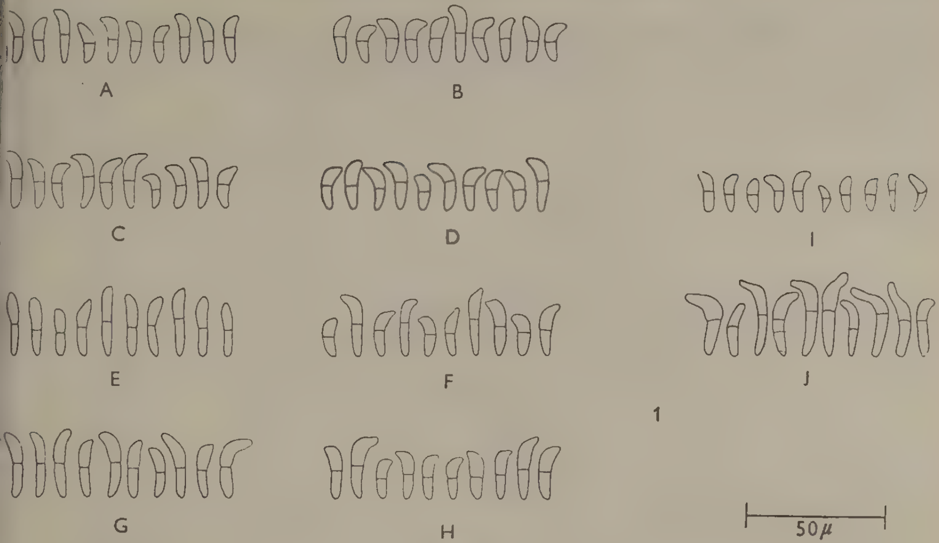

Fig. 1.

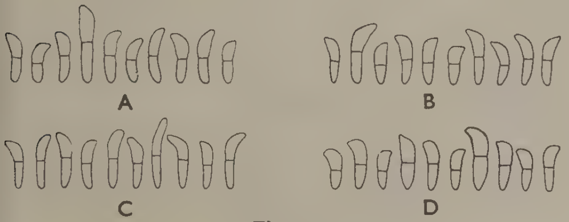

Fig. 2

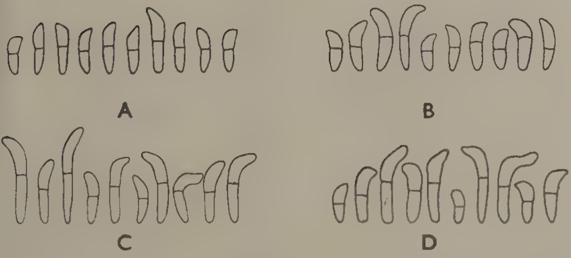

Fig. 3

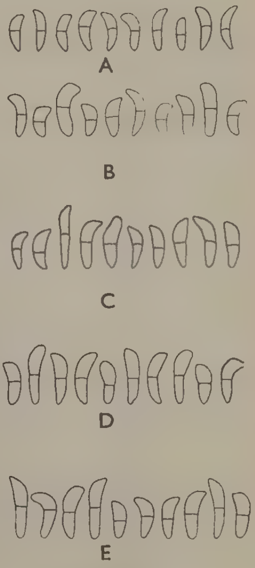

Fig. 4

Text-figs. 1–4. Spores of *Rhynchosporium secalis*

Text-fig. 1. Isolates from barley. A, B, 907; C, D, 856; E, F, 782; G, H, 872; I, 765; J, 781. A, C, E, G, spores from leaves. B, D, F, H, I, J, spores from cultures.

Text-fig. 2. Isolates from rye. A, B, 942; C, D, 888; A, C, spores from leaves. B, D, spores from cultures.

Text-fig. 3. Isolates from *Hordeum murinum*. A, B, 814; C, D, 802. A, C, spores from leaves; B, D, spores from cultures.

Text-fig. 4. Isolates from *Agropyron repens*. A, 806; B, 930; C, 808; D, 919; E, 946. Spores from cultures.

Table 2. *Statistical analysis of spore length in 26 isolates of* R. secalis *from leaves and cultures*

	D.F.	M.S.
Total	2599	
Sources	1	1748·0***
Between hosts	3	246·6**
Within hosts	22	207·5
Between isolates	25	212·2
Sources × hosts	3	23·0
Error (1)	22	50·6***
Sources × isolates	25	47·3***
Error (2)	2548	4·96

** Significant at $P<0{\cdot}01$. *** Significant at $P<0{\cdot}001$.

Source means (μ)

Leaves:	19·05
Cultures:	17·41

Host means (μ)

Spores from leaves			Spores from cultures		
Hordeum murinum	20·14 ± 0·50	*	*Hordeum murinum*	18·69 ± 0·50	*
Barley	18·93 ± 0·23		Barley	17·21 ± 0·23	
Rye	18·93 ± 0·71		*H. leporinum*	17·13 ± 1·01	
H. leporinum	17·22 ± 1·01		Rye	16·93 ± 0·71	

* Difference significant at $P<0{\cdot}05$.

Table 3. *Length of spores from leaves for 26 isolates of* R. secalis (μ)

Isolate no.	Host	Range	Mean	
802	*Hordeum murinum*	16·0–35·3	25·32	
782	Barley	16·7–27·8	22·00	±0·31
872	Barley	16·0–30·0	21·45	
884	Barley	15·0–27·3	21·06	
772	Barley	16·2–25·3	20·98	
888	Rye	16·0–25·8	20·50	
886	Barley	15·0–24·3	19·97	
880	Barley	16·0–24·8	19·37	
860	Barley	13·7–24·3	19·30	
911	Barley	14·5–24·0	19·10	
924	Barley	14·5–22·8	19·03	
937	*H. murinum*	14·2–22·3	18·97	
933	*H. murinum*	14·7–26·5		
889	Barley	14·2–24·5	18·94	
885	Barley	15·0–24·3	18·69	
854	Barley	15·0–25·3	18·49	
864	Barley	13·7–21·8	18·11	
856	Barley	12·5–26·3	18·03	
831	Barley	14·2–21·0	17·95	
954	Barley	12·2–24·5	17·52	
942	Rye	14·0–28·0	17·36	
814	*H. murinum*	13·7–24·0	17·31	
937	Barley	14·0–22·0	17·29	
887	*H. leporinum*	11·5–22·3	17·24	
907	Barley	13·7–19·5	16·53	
921	Barley	12·5–21·5	15·90	

	D.F.	M.S.	
Total	1299	—	
Isolates	25	197·25	(Significant at $P<0{\cdot}001$)
Error	1274	4·91	

Table 2 shows that the spores of *R. secalis* ex *Hordeum murinum* are significantly longer than those from other hosts, whether the spores come from leaves or from cultures, but that no other between-hosts difference is significant. From Tables 3 and 4 it is clear that there are many significant differences between isolates, including differences between isolates from the same species of host plant.

Table 4. *Length of spores from cultures for 35 isolates of* R. secalis (μ)

Isolate no.	Host	Range	Mean	
853	*Hordeum leporinum*	17·5–40·5	26·90	
781	Barley	18·0–31·0	25·00	
802	*H. murinum*	11·2–30·8	20·28	
889	Barley	14·7–28·3	19·66	
886	Barley	14·0–24·8	19·55	
933	*H. murinum*	13·2–27·3	19·21	
837	*H. murinum*	14·0–24·8	18·69	
872	Barley	14·5–28·0	18·62	
946	*Agropyron repens*	13·5–22·5	18·40	
919	*A. repens*	12·5–21·8	17·91	
884	Barley	13·7–25·5	17·87	
782	Barley	13·5–24·3	17·59	
772	Barley	14·5–21·5	17·44	
924	Barley	14·2–23·8	17·40	
880	Barley	12·5–23·5	17·35	
937	Barley	13·0–22·8	17·31	
888	Rye	12·7–23·0	17·19	
887	*H. leporinum*	14·0–20·8	17·14	±0·30
911	Barley	13·0–22·0	17·06	
808	*A. repens*	12·2–23·5	17·04	
954	Barley	12·2–26·3	16·96	
831	Barley	12·7–20·5	16·73	
942	Rye	14·0–22·5	16·67	
854	Barley	13·5–21·5	16·66	
814	*H. murinum*	12·7–24·0	16·59	
864	Barley	13·7–20·0	16·57	
907	Barley	13·0–20·5	16·22	
921	Barley	13·5–22·8	16·21	
856	Barley	13·0–20·0	16·07	
885	Barley	12·7–19·3	16·03	
941	Barley	12·5–18·8	15·87	
860	Barley	12·5–18·5	15·62	
806	*A. repens*	12·2–18·8	14·88	
930	*A. repens*	10·7–21·0	13·73	
765	Barley	9·5–15·8	12·94	

	D.F.	M.S.	
Total	1749	—	
Isolates	34	335·04	(Significant at $P<0·001$)
Error	1715	4·36	

GROWTH IN CULTURE

Behaviour in culture of a selection of isolates of *R. secalis* from different hosts was studied on the following media, in both test-tube and Petri dish cultures: 3 % malt-extract agar; 3 % malt-extract agar containing 20 g. dextrose and 1 g. peptone per l.; potato 2 % dextrose agar (extract derived from 200 g. potato); potato 2 % dextrose agar containing 2 g. asparagine, 0·75 g. magnesium sulphate and 1·25 g. potassium monohydrogen phosphate per l.; Czapek-Dox agar, containing sucrose; Czapek-Dox agar, with sucrose replaced by dextrose; oat meal agar; corn (maize) meal agar; barley leaf agar; potato-carrot agar (extract derived from 20 g. potato and 20 g. carrot).

The cultures were incubated at 20° C., which had been found to be near the optimum temperature for growth. All isolates grew very slowly on all media. A representative isolate, in plate culture, had colonies varying from 15 to 27 mm. diam. after 40 days, according to the medium.

The mycelium was very closely interwoven and compact. In general, little aerial mycelium was produced. The colonies were convex and much puckered or wrinkled, often with conspicuous radial furrows.

Spores were formed on the surface of the colonies. In some isolates they developed freely, and lay in a slimy layer on the surface of the growth. In others they were produced less profusely, and tended to be intermingled with the mycelium, so that they were difficult to remove in quantity. A few isolates sporulated meagrely, and one (780) not at all. Sporulation was greater on media with low soluble nutrient content (e.g. corn meal and potato-carrot agars) than on richer media such as malt and potato-dextrose agars. Spores produced on the richer media were mostly abnormal in shape, compared with spores produced on leaves.

Colonies were pale (off-white, buff or pink) at first, but mostly developed a dark pigment after a few weeks, on the richer media, and old cultures of most isolates were almost black. On media relatively low in nutrients, such as potato-carrot agar, there was less development of dark pigment. Very thin, spreading colonies almost devoid of pigmentation were produced on Czapek-Dox agar and its modification.

Certain isolates were conspicuous in never producing any dark pigment. On malt agar and potato-dextrose agar the colonies usually soon acquired a rich golden colour, which was retained throughout the life of the culture.

On some media there were greater differences in growth from isolate to isolate than on others. On Czapek-Dox agar all isolates formed almost identical colonies. Potato-dextrose agar showed variations between isolates better than any other medium tested. All isolates listed in Table 1 were grown on potato-dextrose agar slopes, and detailed observations were made after 30, 60 and 90 days. Photographs of some of the cultures are shown in Pl. 3. Much variation was evident at the first examination, but the cultures became more alike as they grew older. There was no marked feature distinguishing the cultures from any one host plant, except in the case of those from *Agropyron repens*. Cultures of isolates from *A. repens* had a wider flat peripheral zone than those from other hosts, and the surface was mainly somewhat deeply radially furrowed rather than irregularly wrinkled. No isolate from *A. repens* produced any dark pigment, the colour varying from pale to deep buff.

DISCUSSION

The inoculation experiments reported above prove conclusively that British isolates of *Rhynchosporium secalis* exhibit physiologic specialization. So far as isolates from barley, rye and *Hordeum murinum* are concerned, my results accord with those of Caldwell (1937). Sarasola & Campi (1947) stated that *R. secalis* isolated from barley in Argentina attacked not only that host but also certain grasses, including *Festuca elatior* and *Bromus catharticus*, but my attempts to infect these two species with isolates of *R. secalis* from England and from Argentina were unsuccessful. No explanation can be offered for the divergence between the strict host specialization observed by Caldwell (1937) and myself, and the absence of specialization reported by most workers in Europe.

In the present work it has not been possible to infect *Agropyron repens* with isolates of *R. secalis* from that species. These isolates did not sporulate abundantly in culture, but sporulation was at least as profuse as in some isolates from barley, which produced infection of barley. The spores of *Agropyron* isolates were tested in germination experiments against those of isolates from other host plants and were found to behave comparably. Further work is necessary to find the conditions for infection of *A. repens*. I have been unable to find infected material of *Bromus* spp., reported by others (e.g. Brooks, 1928) as hosts of *R. secalis*. The position concerning specialization within *R. secalis* is therefore in doubt with regard to isolates from at least two hosts. The fact that the five available isolates of *R. secalis* from *A. repens* failed to cause infection under the same conditions as those for all the successful inoculations suggests that the physiology of these isolates may be different from that of isolates from other hosts and may perhaps be regarded tentatively as evidence of physiologic specialization.

Caldwell (1937) measured spores from each race which he described, and reported differences in mean spore length and width between some races, for spores produced in culture. Since the measurements of only one sample of each race were presented, it would seem that he measured spores of only one isolate from each host plant. It is to be inferred from the results of the present investigation that the variation in spore size which Caldwell found was a reflexion of between-isolates differences rather than between-hosts differences.

Caldwell also compared one representative isolate of each of five physiologic races of *R. secalis* in culture, and distinguished two general types of growth: races from rye, *Bromus inermis* and *Elymus canadensis* grew out from the inoculum in a uniform radial manner, while races from barley and *Agropyron repens* heaped up to form irregular masses of mycelium and conidia. He stated that the characters described were relatively constant for each race. This has not been my experience. I did not find two distinct types of growth manifested by the isolates studied, and with the exception of isolates from *A. repens* it was not possible to distinguish cultures from any one host plant.

In Argentina, the presence within *R. secalis* of groups of biotypes specialized to particular cultivars of barley was found by Sarasola & Campi (1947), and the existence of a similar kind of specialization in North America has been implied by Riddle & Briggs (1950). This second level of specialization has not yet been shown to exist in Britain. Sarasola & Campi would term the 'physiologic races' of Caldwell (1937) 'varieties', reserving the term 'physiologic race' for a subdivision of the fungus species distinguished by its pathogenicity to barley cultivars. 'Variety' does not appear to be a suitable term to employ in this connexion, however, in the absence of distinctive morphological characteristics, and '*forma specialis*' is to be preferred, using 'race' for a group of biotypes distinguished on pathogenicity to particular cultivars of barley (or other host).

I am grateful to all who have sent material for study, especially to Miss June V. Ives and Mr E. T. Roberts of the N.A.A.S., and to colleagues for assistance in various ways and for valued advice and criticism.

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EXPLANATION OF PLATES 2 AND 3

PLATE 2

Rhynchosporium secalis

Fig. 1. Symptoms on barley. ×$\frac{1}{4}$.
Fig. 2. Symptoms on barley. ×$\frac{2}{5}$.
Fig. 3. Symptoms on rye. ×$\frac{4}{5}$.
Fig. 4. Symptoms on *Agropyron repens*. ×$\frac{3}{5}$.
Fig. 5. Symptoms on *Hordeum murinum*. × 1.
Fig. 6. Symptoms on *H. murinum*. ×$\frac{3}{4}$.

PLATE 3

Cultures of *Rhynchosporium secalis* on $\frac{3}{4}$ in. test-tube slopes of potato 2 % dextrose agar after 90 days at 20° C.

Fig. 1. Isolates from barley (left to right, top row first: nos. 765, 772, 781, 831, 854, 856, 860, 864, 872, 880, 907, 911, 921, 924, 937, 941, 954, 889, 886, 885, 782, 884, 780).
Fig. 2. Isolates from rye (left to right: nos. 942, 888).
Fig. 3. Isolates from *Agropyron repens* (left to right: nos. 806, 808, 919, 930, 946).
Fig. 4. Isolates from *Hordeum murinum* (left to right: nos. 802, 814, 837, 933).
Fig. 5. Isolates from *H. leporinum* (left to right: nos. 853, 887).

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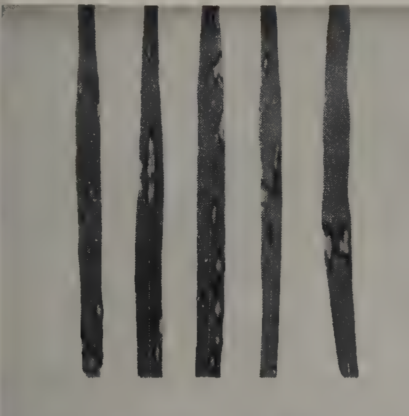

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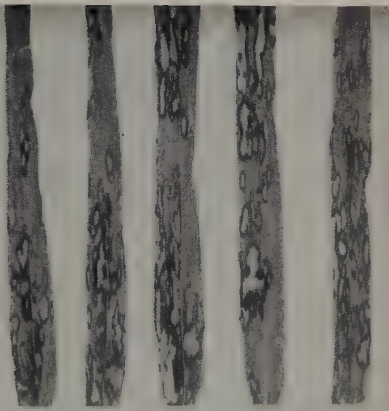

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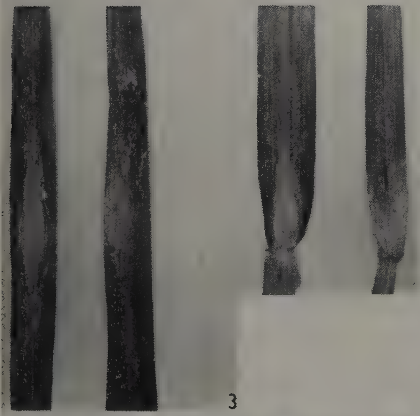

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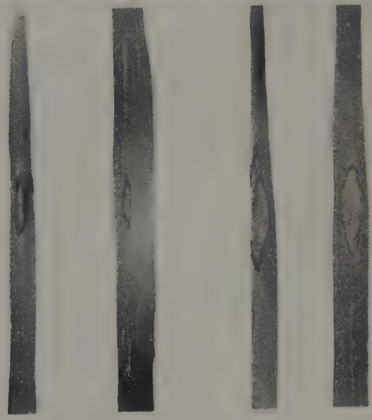

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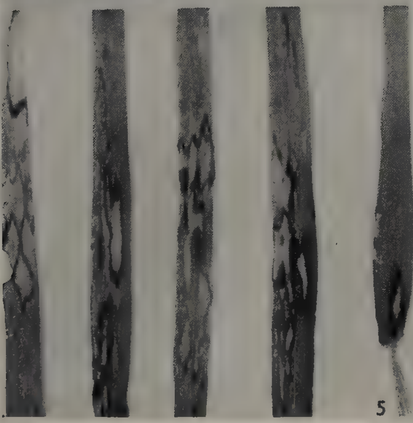

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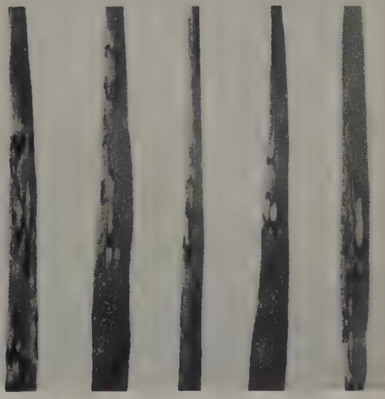

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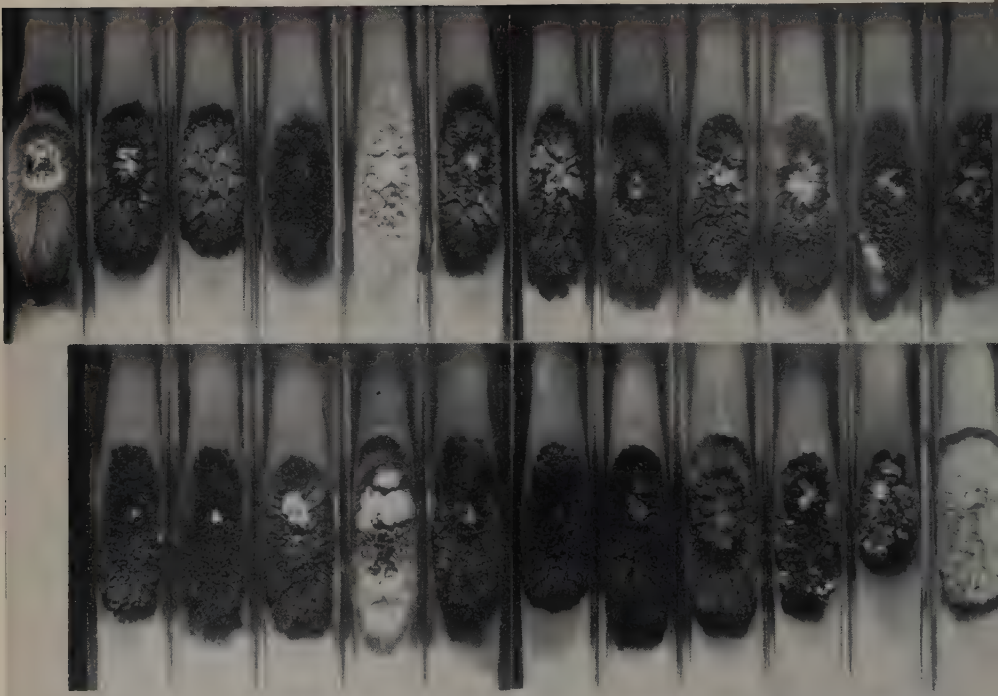

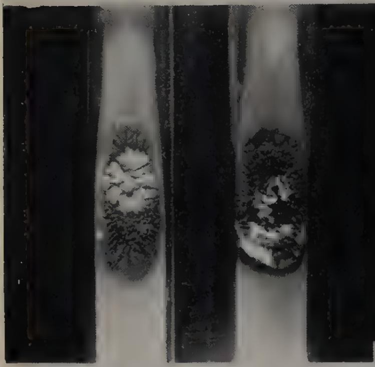
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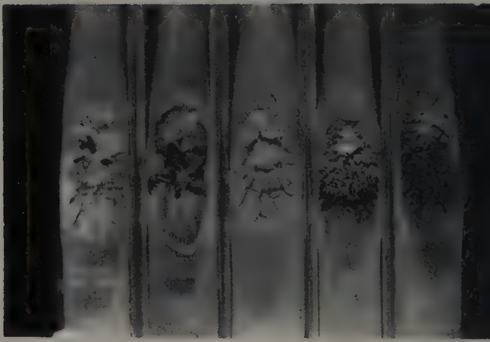

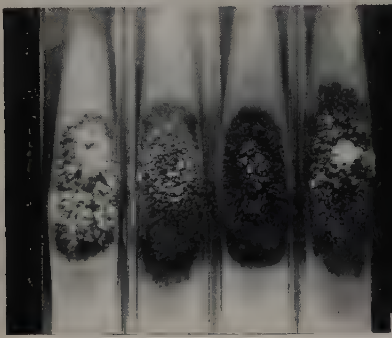
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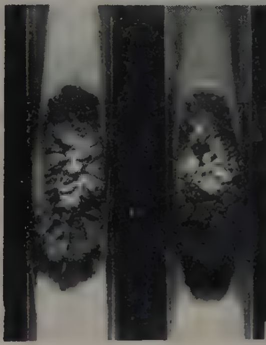
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Trans. Brit. mycol. Soc. **41** (1), 109–114 (1958).

AQUATIC HYPHOMYCETES FROM UGANDA AND RHODESIA

By C. T. INGOLD

Birkbeck College, University of London

(With 5 Text-figures)

Twelve known species of aquatic Hyphomycetes are recorded from Uganda and seven from Rhodesia. In addition, a number of previously undescribed types of branched hyphomycete spores are figured from Uganda. One type of spore is shown to have close affinities with spores of *Tripospermum* and *Campylospora*. This spore-type is believed to be closely connected with certain spores of curious form previously seen in Britain.

Recently the presence of spores of aquatic Hyphomycetes was reported in the foam and scum from a stream in Nigeria (Ingold, 1956). A further visit to Africa in 1957, not directly concerned with mycology, gave me an opportunity of studying these fungi in Uganda and Southern Rhodesia. Although little time was available and only a few observations were made, I feel these should be put on record, if only to encourage others to make a thorough investigation.

In Uganda collections were made from three localities near Kampala: the river just below the Sezibwa Falls, a stream near Namulonge Cotton Research Station and a stream in the Mpanga experimental forest. The single sample from the first locality consisted of brownish foam at the edge of the river just below the waterfall. The sample from the second locality was typical scum held back by a barrier of twigs and leaves in mid-stream. The principal sample from the Mpanga stream was composed of wringings from submerged leaves. In addition, from this stream skeletonized leaves of broad-leaved trees were collected, placed in shallow water in Petri dishes and later searched for aquatic Hyphomycetes under the low power of the microscope.

In Southern Rhodesia collections from two localities were examined. One consisted of brownish foam from a stream 15 miles east of Salisbury; the other of wringings of submerged decaying leaves from a stream in the Inyanga district on the eastern borders of the country.

Table 1 records the fungi found. Thirteen clearly referable to described species are involved. This contrasts with my observations in Nigeria where only three known species were identified from the stream spora. It is interesting to note that all three of these, *Actinospora megalospora*, *Campylospora chaetocladia* and *Triscelophorus monosporus*, are here recorded from Uganda and the last two from Southern Rhodesia.

As in scum and foam samples examined from other places, the spora is dominated largely by branched spores (mainly tetra-radiate) and by long filiform or vermiform spores. Amongst the branched spores there occurred some which are worthy of mention, but which do not appear to belong t described species.

The spores shown in Fig. 1 were fairly common in scum from th Namulonge stream and in wringings derived from submerged leaves fro the stream in Mpanga Forest. The hyaline spore consists of a short, non-septate stalk from the slightly swollen apex of which there arise 6–8 long

Table 1. *Aquatic Hyphomycetes from Uganda and Southern Rhodesia*

	Uganda			Southern Rhodesia	
Species	Mpanga Forest	Sezibwa Falls	Namulonge	Stream east of Salisbury	Inyanga
Actinospora megalospora	.	×	×	.	.
Alatospora acuminata	.	.	×	×	.
Anguillospora longissima	⊗	.	.	.	.
Articulospora tetracladia	.	.	×	.	.
Campylospora chaetocladia	⊗	.	.	×	.
Clavariopsis aquatica	⊗	.	.	.	.
Flagellospora curvula	⊗	.	.	×	.
Lunulospora curvula	⊗	.	.	×	.
Tetrachaetum elegans	.	.	.	.	×
Tetracladium marchalianum	⊗	.	.	×	.
T. setigerum	.	.	×	.	.
Tricladium angulatum	×	×	×	.	.
Triscelophorus monosporus	⊗	.	.	×	×

Note. Those species marked thus ⊗ were recorded not only as isolated spores but also as spores attached to conidiophores arising from submerged decaying leaves.

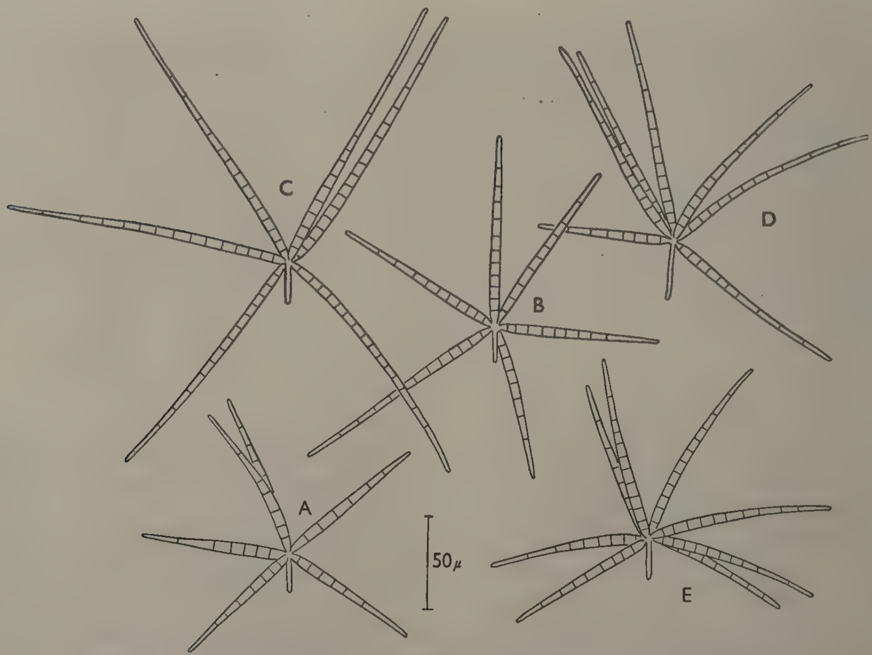

Fig. 1. Spores belonging to an unknown genus. A and B, from Mpanga; C, D and E, from Namulonge.

(80–150 μ × 5–7 μ), septate arms which taper towards their free ends. Spores of this type probably belong to an undescribed genus.

A second striking example was the type illustrated in Fig. 2. This occurred in the foam below the Sezibwa Falls. The spores were relatively enormous and occurred in considerable number. The hyaline spore consists of four straight, divergent, septate arms each 200–300 μ long and 20–25 μ wide. The only other spore of an aquatic hyphomycete comparable in size with this is that of *Actinospora megalospora*, which was also very abundant in the same foam sample. Structurally the spore seems to consist essentially of a main axis from the end of which the other three arms spring, each being somewhat constricted where it joins the main (probably first-formed) axis. In structure, but not in size, the spore shows close agreement with that of *Articulospora tetracladia*.

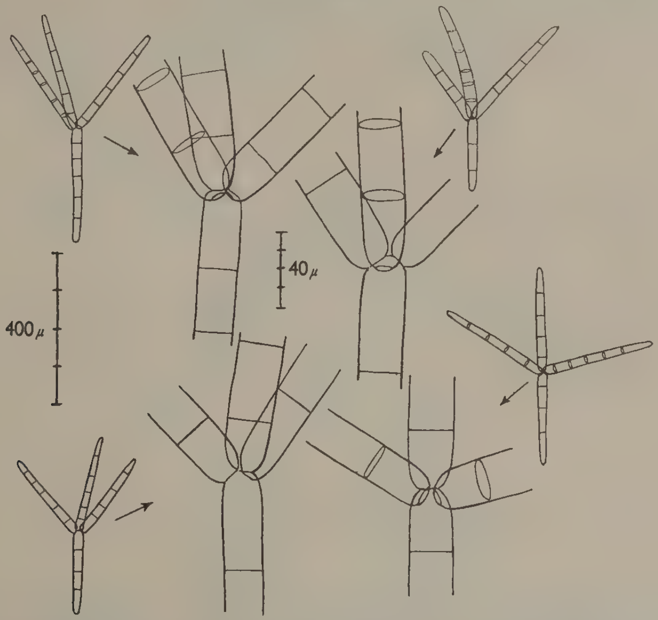

Fig. 2. Four spores of an unknown species from Sezibwa Falls drawn under low power. High-power details of the central region of each spore are also shown.

In the Namulonge scum a curious spore (Fig. 3A) was found. It consists of six long, hyaline, septate arms forming a plume-like tuft. It is made up of a main axis from near the base of which two laterals arise. The lower of these itself gives off two laterals and the upper a single one. Again an undescribed genus would seem to be involved.

The hyaline spore shown in Fig. 3B, also from Namulonge, is again of a curious type. From a non-septate stalk region two branches arise, each of which almost at once dichotomizes again so that four longish, septate arms are produced.

The tri-radiate type of spore illustrated in Fig. 3C is distinctive. All the arms are constricted at intervals so that they look like chains of sausages.

The hyaline spore would appear to consist of a main axis with two laterals arising near the base. Possibly this type may belong to a species of *Triscelophorus* in which the fourth arm has failed to develop.

Fig. 3D shows spores, abundant in the foam from the Sezibwa Falls, which agree perfectly with those illustrated from a Nigerian stream (Ingold, 1956, fig. 1 E). As previously suggested these may well belong to a species of *Clavariopsis*.

The spores illustrated in Fig. 3E, F were quite frequent in wringings from leaves from the Mpanga stream and were also seen attached, in various stages of development, on submerged skeletonized leaves from the same locality. Exactly similar spores were also very abundant in the foam sample from the stream 15 miles east of Salisbury. It is fairly clear from the structure of the spore that a species of either *Clavariopsis* or *Heliscus* is involved. My observation on the attached spores confirm this, for the relatively thick clavate arm develops first and the three (very occasionally four) other arms arise simultaneously from its crown and grow out together. Further, I was almost satisfied that the spore is borne on a phialide and, if that is so, the fungus is a species of *Heliscus*.

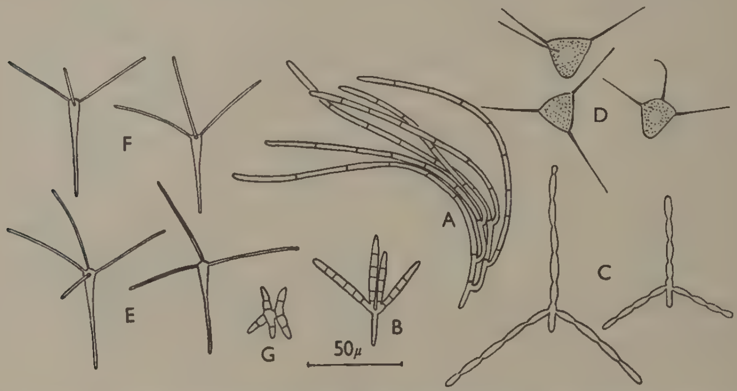

Fig. 3. A–E and G, various spore-types from Namulonge scum; F, spores from scum on stream near Salisbury, Southern Rhodesia.

Spores of *Campylospora chaetocladia* (Ranzoni, 1953) were found in abundance both in Uganda and in Southern Rhodesia. On leaves from the Mpanga Forest stream I saw this species for the first time growing on its natural substrate and was able to confirm Ranzoni's account of spore development (Fig. 4).

A small spore from the Namulonge scum (Fig. 3G) is of some interest. It clearly belongs to the genus *Tripospermum* and, indeed, differs from such a species as *T. myrti* only in having all its arms more or less in one plane. It has recently been pointed out (Ingold & Cox, 1957) that *Tripospermum* and *Campylospora* may be considered closely allied genera and, perhaps, should be merged in one genus. The agreement between *Campylospora* and the spore shown in Fig. 3G is at once evident on comparing it with the development stage of *Campylospora* illustrated in Fig. 4D. If the spore

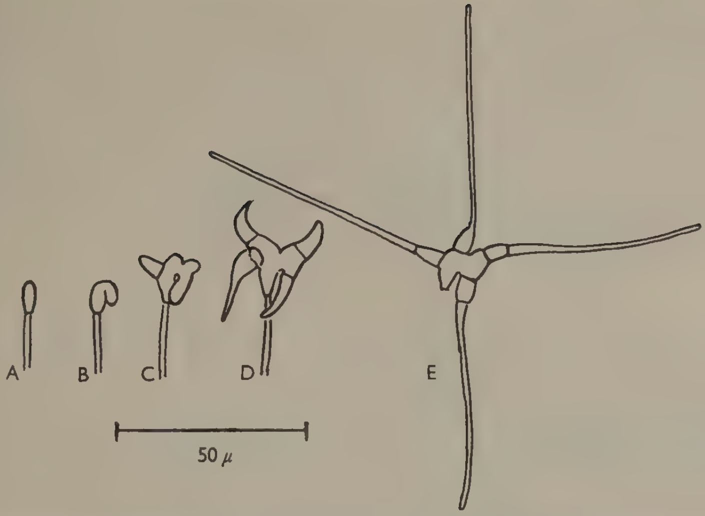

Fig. 4. *Campylospora chaetocladia.* A–D, conidiophores with conidia at progressively later stages of development. E, liberated spore. From Mpanga Forest.

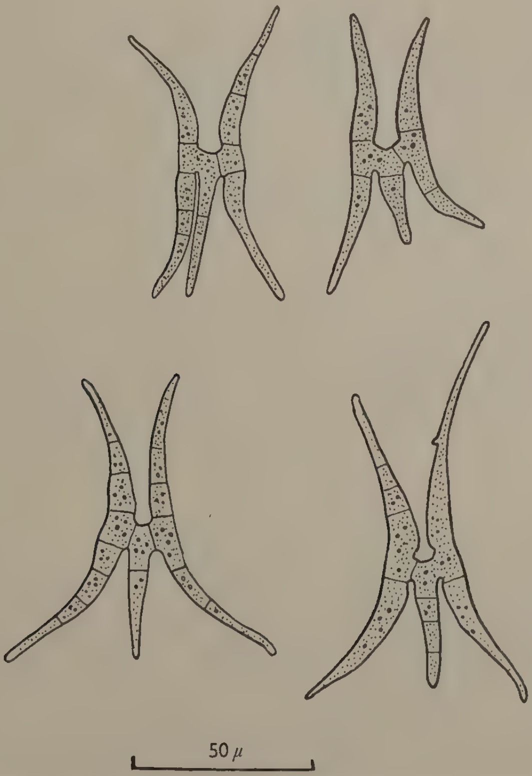

Fig. 5. Spores of an unknown fungus from the scum of a stream in Leicestershire.

shown in Fig. 3G is correctly assigned to *Tripospermum*, it is clear that another kind of spore seen originally in scum from a stream in Leicestershire (Ingold, 1942; fig. 1, no. 18) is also referable to that genus or possibly to *Campylospora*. Some of these spores are illustrated in Fig. 5.

My grateful thanks are due to Dr Edna Lind of Makerere College, Uganda, and to Prof. A. S. Boughey of the University College of Rhodesia and Nyasaland for their kindness in transporting me to suitable collecting grounds and in giving me facilities in their laboratories.

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THE DEVELOPMENT OF LOOSE SMUT (*USTILAGO TRITICI*) IN SUSCEPTIBLE VARIETIES OF WHEAT, AND SOME OBSERVATIONS ON FIELD INFECTION

By C. C. V. BATTS* and ANN JEATER

National Institute of Agricultural Botany, Cambridge

(With Plate 4 and 3 Text-figures)

Infected seed of the susceptible wheat varieties Hybrid 46 and Vilmorin 27 was obtained by artificial inoculation. In an infected embryo, loose smut mycelium is present in the scutellum and growing point region. When germination of the seed commences, the fungus also becomes active and the mycelium is carried upwards by the elongation of the epicotyl. This elongation carries the node of the first foliage leaf to a position near the ground level. This node, which contains mycelium, becomes the first crown node.

Development then takes place in the crown, until by about April the entire adult plant is laid down in the crown region. The nodes, internodes, and ears of each tiller can be recognized within the leaf sheaths, but their combined length is only about ¼ in. Loose smut mycelium permeates the whole structure.

Expansion of the stems begins from below upwards, the lowest internode of each tiller being the first to elongate. The mycelium already present in the ear is carried up with it, and on emergence the ear is completely replaced by spores.

Variations in the amount and forms of infection are discussed. Field observations and a time-of-sowing experiment have shown that loose smut infection is not influenced by environmental conditions after sowing.

Batts (1955*a*) dealt with the path of loose smut infection (*Ustilago tritici* (Pers.) Rostr.) in the developing wheat grain. The development of the fungus in the germinating seed, in the subsequent seedling and in the adult plant appears to have received little detailed attention. The fungus is usually described as 'keeping pace with the growing point', but no details have been found in the literature.

MATERIALS AND METHODS

Infected seed of susceptible wheat varieties Hybrid 46 and Vilmorin 27 was obtained by artificially inoculating ears by the partial vacuum method. For examination at the early stages of development, seed was sown on moist filter-paper in Petri dishes, and, for examination of seedlings and older plants, seed was sown in the field in autumn. For investigation of the position of the mycelium, the plants were carefully lifted and washed in water where necessary, and the appropriate portions removed for staining in cotton blue in lactophenol.

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* Now at Imperial College of Science and Technology, Field Station, Silwood Park, Sunninghill, Berks.

The terminology used in describing the developing wheat plant is taken from Percival (1921) and McCall (1934).

OBSERVATIONS

In an infected wheat embryo, the loose smut mycelium is present in the scutellum and growing point region. The mycelium, which is mainly intercellular, varies in amount from a few strands to thick wefts occupying much of the scutellum. There is no evidence of the fungus having any adverse effect on the host cells.

When germination of the grain commences, the fungus also becomes active. Five days after sowing in a Petri dish, when the coleoptile and radicle have largely emerged from the ruptured seed coat, mycelium is found ramifying among the short spirally thickened cells behind the growing point, and even in the meristematic tissue itself. No mycelium was present in the vascular tissue, leaf-base tissue or root initials.

The primary stem of the wheat embryo within the grain is extremely short and consists of the hypocotyl or portion below the point of insertion of the scutellum, and the epicotyl or axis of the plumule. As growth proceeds the coleoptile enlarges and the first foliage leaf appears. The roots increase in length and the epicotyl, or axis above the point of attachment of the coleoptile, grows upwards. The second foliage leaf pushes out. Elongation of the epicotyl continues until the node of the first foliage leaf reaches a point near the soil surface. Elongation below this node then ceases. Adventitious roots are formed at this node, which then becomes the first node of the crown. The region between the coleoptile attachment and the first crown node is called the subcrown internode, or is occasionally referred to as the mesocotyl.

Mycelium is present in the epicotyl and associated nodes, and elongation of the epicotyl carries the mycelium upwards through the soil. Mycelium is therefore present in the first crown node. A few very short bits of mycelium are occasionally found in the subcrown internode and they have apparently been carried there during the rapid development of the cells. These isolated bits of mycelium presumably play no further part in the development of the pathogen within the host.

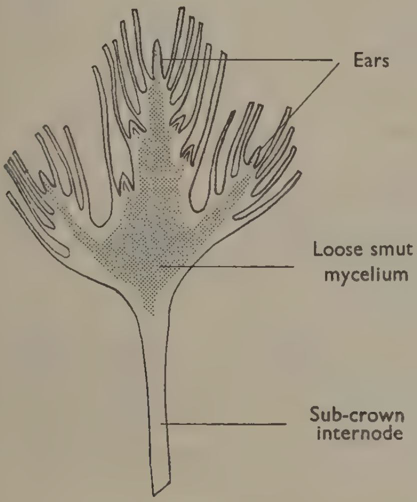
The scutellum still retains its identity even when the crown node is present and, in susceptible varieties, when mycelium was present in the scutellum it could always be found also in the crown node.

In one plant of Hybrid 46 examined 4 weeks after sowing, mycelium was found in the root initials. This has only been seen in this one plant, out of the many examined.

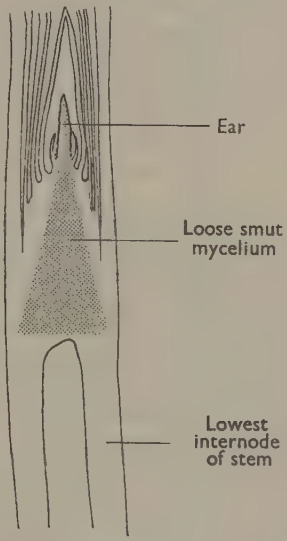
When the first crown node has reached the soil surface, the lowest foliage leaves soon appear, and adventitious roots are formed. Considerable development of the crown node then takes place. Branches appear on each side of the primary stem, and each of these branches then gives rise to two further branches, and the tillers are thus produced. The crown region is therefore made up of a number of successive nodes and internodes. The latter lengthen very little, and the whole crown structure is much compressed and shortened.

The mycelium also continues growing in the crown and passes from the original crown node into the bases of the tillers.

Neither the primary axis nor any of the branches arising during the tillering period continue to produce lateral vegetative shoots indefinitely, for sooner or later inflorescences originate at the apices of the branches, and lateral bud formation ceases. The first indication of spikelet development is the appearance of double ridges on the growing point (Bonnett, 1936). The hyphae then grow into the inflorescences from the nodes below.



Text-fig. 1



Text-fig. 2

Text-fig. 1. Semi-diagrammatic longitudinal section of crown region of an infected seedling plant. (Section based on Percival, 1921.)

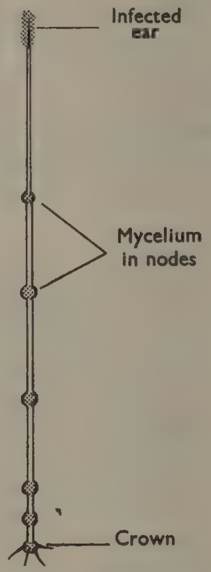
Text-fig. 2. Semi-diagrammatic longitudinal section through an infected tiller with stem elongation just beginning. (Section based on Percival, 1921.)

In the normal sequence of events, therefore, in wheat sown between October and the end of March, the entire adult plant is laid down by about April. The nodes, internodes and ears of each tiller can be recognized within the leaf sheaths but their combined length is only about a quarter of an inch. On an average, six nodes are present in each tiller. Loose smut mycelium permeates the whole structure (Text-fig. 1).

Growth advances from this stage with increasing rapidity until the straw reaches its full height and the ears emerge from the uppermost leaf sheath by the elongation of the last internode or peduncle.

The expansion of the stems begins *from below upwards*, i.e. the lowest internode of each tiller is the first to elongate (Text-fig. 2). The growth of this is followed more or less regularly by that of the succeeding internodes, in numerical order to the sixth or uppermost. Growth ceases in the same order, the lower internodes reaching their full length some time before the upper ones. In an infected plant, therefore, the mycelium already present in the ear is carried up with it—it does not have to grow up behind the ear as is usually thought to happen. In the fully expanded stem, mycelium can be found in each node, but not in the internodes (Text-fig. 3)—expansion of the cells of the internodes probably being too rapid for the growth of the mycelium.

Spore formation takes place some time before the ear emerges. Ears about an inch long appear brownish in colour and some spores are already present together with large amounts of mycelium. As the ear continues to expand, spores are formed in increasing numbers, until on emergence the ear appears as the typical black spore mass.



Text-fig. 3. Diagrammatic representation of a mature wheat tiller with leaves and sheaths removed showing position of loose smut mycelium.

Differences in amount of infection

Not all the tillers of an infected plant necessarily produce smutted ears. As indicated previously, the amount of mycelium present in infected embryos varies. In those where there is comparatively little, there would be correspondingly little in the crown node, and only a proportion of the tillers would become infected. There is known to be a correlation between the percentage of plants attacked and the severity of attack of the individual plants, i.e. the proportion of diseased tillers (Oort, 1947; Batts, 1955*b*). In very susceptible varieties, a greater proportion of tillers are infected. Conditions appear most favourable for mycelial development in very susceptible hosts, but what these conditions are is not known. In fairly resistant varieties, although the fungus can develop to a certain extent, the amount of mycelium, and hence the number of infected tillers, is less.

There are certain varieties in which only small portions of the glumes are infected—the grain developing normally. Here conditions would seem to be highly unfavourable for the fungus. At the other extreme, in some very susceptible varieties an occasional plant is seen where not only are the ears completely infected, but spores develop on the flag leaves and sheaths also. The spore masses are then in lines (Pl. 4, fig. 1). On one occasion, the flag leaf and its sheath were almost completely covered with spore masses, and the second leaf down and its sheath also bore a few spore-producing areas (Pl. 4, fig. 2). In some other varieties, the flag leaves of infected plants are yellow in colour.

Yet another variation is seen when only a part of the ear becomes infected. In the greatest number of cases it is the bottom portion which is infected, i.e. the upper half or third produces quite normal spikelets. In these cases it would appear that the mycelium had only reached part way up the rachis during the development of the ear. No mycelium has been found in the portion of the rachis bearing healthy spikelets. It is interesting to record that out of the scores of thousands of infected ears seen during the past few years, two ears have been found where only the upper spikelets were replaced by spores.

Observations of previous workers

There are many references to the presence of loose smut mycelium in the embryos of infected grains (see Popp, 1951), but the presence of infection in seedlings and older plants has received much less attention. Popp (1951) found that when infection was present in the crown node, it was also in the scutellum of the same seedling. Koursanoff (1926) found mycelium in the stems, from whence it permeated the ears. He also found a certain amount in the leaf sheaths, and another Russian worker (Klushnikova, 1928) found that the greatest amount of infection was present in the coleoptile and in the lowest of the apical leaves, but mycelium was present also in the nodes and internodes. Sabourova (1939) observed infection of developing ears in plants with the third or fourth node present.

Some interesting experiments were made by Vanderwalle (1942). He removed the first nodes (crown node?) of infected seedlings, carefully planted them in warm moist soil to encourage root formation, and then transplanted them in the open so that they developed normally. These plants produced smutted ears in the normal way. From the experiments described in this paper, this would be expected. He then, however, made a few trials with second nodes, but of the plants which survived (no numbers are given) none produced infected ears. It would have been interesting to know if the seeds of these plants were actually infected. Vanderwalle's interpretation of these observations was that the mycelium in the growing point of the embryo develops rapidly at germination, but gradually loses ground as the host develops, and becomes out-distanced, and only reaches the top of the stem again when the spikelet primordia differentiate.

In the susceptible varieties tested at Cambridge, the presence of the fungus had very little effect on the morphology of the plant, except of course for the ear and an occasional leaf. Oort (1944), however, has reported what he considers to be severe hypersensitivity in certain wheat varieties, grown in the glasshouse after inoculation with particular races of the fungus. The most striking symptom is a growth inhibition, shown by a shortening of the first three leaves, sometimes accompanied also by chlorotic stripes and spots, and malformation of the leaves. Many plants showing these symptoms do not reach maturity, but those which do so produce healthy ears. A cytological investigation of these phenomena would be interesting. Two other forms of abnormality have been described (Ohms & Bever, 1955) in the varieties Wabash and Kawvale. One took the form of abnormal internode elongation in both varieties, and the other was expressed as the death of seedlings of Wabash (inoculated with race 11) between the third and fifth foliage leaf stage. Except for discoloration of the flag leaves in some varieties, no such adverse effects have been seen on the varieties so far handled. Discoloration of leaf sheaths and exposed portions of culms of infected plants has been reported (Tingey & Tolman, 1934).

Shevchenko (1955) found that a selection, over a period of five years, of spring wheat seed having large size, high specific gravity, and good germination, resulted in increased resistance to loose smut. It is difficult to understand the theory behind this, since infection takes place at about the same time as pollination, i.e. before the seed is formed, and infection does not reduce the size of the seed. However, Niemann (1955) has repeated this work and was unable to obtain the same result.

In experiments on the effect of tillering on loose smut infection in wheat, Milan (1939) found that rate of sowing had virtually no effect on the percentage of plants infected, but very greatly affected the percentage of ears that became diseased. This may be explained by the results given earlier. In any infected plant, a certain amount of mycelium is present. If only few tillers develop, all may become infected. If, however, a large number of tillers develops, the available mycelium may be able to infect only a proportion of them.

Field observations on infection

The idea is frequently expressed by seed merchants and others that the amount of loose smut which appears in a crop depends not only on how much infection is present in the seed, but also to a large extent on the time of sowing, nature of soil, etc. Sometimes early sowing, at other times late sowing, is said to give least infection. If the theory were consistent, it could be utilized as a measure of control.

During the past few years, several cases of alleged differences in infection between fields sown with the same stock of seed have been investigated. A 'stock' is a bulk of seed which originally came from one field. Careful counts, by two people working as a pair, in a number of 24 × 1 yard quadrats as suggested in the booklet on the Cereal Field Approval Scheme (N.I.A.B., 1956), failed to find any such differences. Infected ears in a crop are not easily seen, particularly when most of the spores have blown away and little remains but the rachis. Mistakes are easily made, and if, as so often happens, there is a breeze, the counting is even more difficult. To obtain figures for infection, the tiller population of the crop also requires to be determined. The figure for the tiller population is usually determined by counting the tillers in at least ten randomly chosen yard lengths of the crop.

When the counts of smutted ears and tillers are made carefully, it is surprising how closely infection figures for different fields sown with the same stock of seed compare (Table 1), especially when one considers what a minute proportion of the acreage is actually examined. The Eclipse wheat (Table 1) was grown on farms in Hunts and Beds, and the Hybrid 46 on farms in Essex. At least ten quadrat counts of infection, and ten yard-length tiller counts were made in each field.

To obtain further information on the effect of sowing seed at different dates, a number of ears of Bersee wheat were artificially inoculated in the summer of 1955. The ears were threshed separately and the grain from each ear was kept in a separate packet. Five ear-rows were then sown in the N.I.A.B. Trial Ground at Cambridge on six different dates (Table 2). When the ears emerged in the summer of 1956, the number of infected and healthy plants in each row was counted, together with the number of infected and healthy tillers on each infected plant. The fifth row of the 3 January sowing was completely healthy, probably due to collecting an uninoculated ear by mistake.

Table 1. *The amount of loose smut in two wheat varieties in 1955*

In each case a number of fields was sown with the same stock of seed.

Seed stock	Field	Infected ears/acre	Mean no. tillers/yard	Tiller population/acre (to nearest 1000)	Amount of smut
Eclipse	1	80	66·4	1,652,000	1 ear in 20,650
	2	120	92·7	2,307,000	1 ear in 19,225
	3	60	45·6	1,125,000	1 ear in 18,750
	4	100	62·8	1,563,000	1 ear in 15,630
Hybrid 46	1	2280	82·5	2,053,000	1 ear in 900
	2	1060	59·6	1,483,000	1 ear in 1,398
	3	1320	54·2	1,349,000	1 ear in 1,022
	4	1880	68·4	1,702,000	1 ear in 905
	5	1940	68·6	1,707,000	1 ear in 879
	6	2120	62·4	1,553,000	1 ear in 732

Several interesting features emerge from the results (Table 2). Except for the last sowing which was obviously too late for the variety—the plants remained partly grassy—the amount of infection in each plot was virtually identical. The percentage number of plants infected in each plot remained constant at each sowing date, and it is also interesting to note that the percentage of infected ears on infected plants was similar in each case.

While there was a certain amount of variation in the percentage of plants infected in each row of any one sowing date, the proportion of ears infected (penultimate column) varied very little between rows. Even in the 'grassy' plot, the same proportion of the ears which did emerge were infected. Time of sowing had therefore no effect whatsoever on the amount of infection in these plots. This is particularly interesting as the weather was extremely variable during the months of sowing. For example, the December temperatures were about 4° above average, while the February temperatures were about 10° F. below average.

DISCUSSION

The usual way of describing the development of loose smut in the growing plant is that 'it keeps pace with the growing point'. One is led to imagine the mycelium growing inside the stem, and that conditions of rapid growth of the stem would cause the fungus in turn to increase its activity, so that it did not get 'left behind'. The present work has shown that, on the contrary, the fungus is in the right place from the beginning. Mycelium is present in the growing point of the embryo in the dormant seed and is then carried up to the crown node, near soil level, by the elongation of the

Table 2. *Percentages of* (A) *plants infected,* (B) *smutted ears on infected plants, in plots of inoculated Bersee seed sown on six dates, 1955/56*

		(A) Plants				(B) Ears			
Sowing date	Row no.	No. plants in row	No. plants infected	% infected/row	% infected/plot	No. ears on infected plants in row	No. ears smutted	% smutted ears/row	% smutted ears on infected plants/plot
27 Oct.	1	14	10	71	57	103	41	39	36
	2	29	14	48		125	46	36	
	3	16	11	68		90	29	32	
	4	22	12	54		95	31	32	
	5	22	12	54		147	60	40	
23 Nov.	1	32	18	56	61	112	40	35	37
	2	16	13	81		121	55	45	
	3	19	11	58		97	33	34	
	4	18	12	66		115	42	36	
	5	20	9	45		82	29	35	
3 Jan.	1	10	6	60	56	49	14	28	31
	2	15	6	40		59	23	38	
	3	14	7	50		45	11	24	
	4	19	14	73		104	35	33	
2 Feb.	1	24	19	79	58	131	44	33	34
	2	19	11	57		62	21	34	
	3	29	13	44		87	28	32	
	4	28	16	57		82	30	36	
	5	23	12	52		97	35	36	
9 Mar.	1	25	19	76	59	122	53	43	38
	2	23	13	56		79	26	33	
	3	26	9	34		53	19	36	
	4	20	9	45		63	24	38	
	5	30	25	83		159	64	40	
6 Apr.	1	24	11	45	37	37	14	37	36
	2*	18	5	27		26	9	34	
	3	14	5	35		26	11	42	
	4	19	7	36		40	14	35	
	5	21	9	42		52	17	32	

* Plants remained partly 'grassy'.

epicotyl. The fungus permeates the tissues of the crown and enters the initials of the inflorescences as they are formed. As the young ear is later carried upwards by the elongation of the lowest internodes, the fungus is taken up with it and the mycelium increases as the ear develops, until on emergence the ear tissues are replaced by spore masses.

Some workers have unfortunately generalized on cereal smuts as a whole, and have applied work on the effects of environmental conditions on the contaminating smuts to interpretation of the smuts that infect the grain in the truest sense. In bunt and in covered smut of barley, for example, it has been shown that external conditions do affect the amount of disease in the crop (Jones & Seif-el-Nasr, 1940). This is perfectly understandable. In these diseases, the seed is only contaminated, and the fungus has to enter the young seedling. There is therefore a weak link in the life cycle at this stage, and the fungus may fail to become established in the seedling. There is no such weak link in the case of loose smut of wheat (or of barley), as infection is established from the start. The same authors expressed the opinion that 'it is scarcely to be expected that an internal infection like loose smut would be greatly influenced, if at all, by external conditions at planting time'.

In his review of the effects of environment on cereal smuts, Tapke (1948) found that much of the information on the effects of various soil conditions on loose smut of wheat and barley was not clear cut, and Butler & Jones (1949) make much the same comment.

Neill (1925) found that the incidence of loose smut was unaffected by conditions of temperature, rainfall, or date of sowing, but depended only on the amount of infection in the seed. Simmonds (1946) found a close correlation between the percentage of infected embryos and the percentage of smutted plants in one variety of wheat examined, and Popp (1951) also found this close correlation in susceptible varieties. There are a few recorded cases where infection died-out during plant development (Popp, 1951; Ohms & Bever, 1955), but this occurred in resistant varieties, and forms of resistance to the disease will be discussed in a further paper. Oort (1940), however, found that low temperatures during the first stages of development of the plant may prevent the manifestation of loose smut.

Vernalization has in most cases been found to have no effect on the incidence of loose smut (Hanna, 1936; Bever, 1943), but Lasser (1938) found that it greatly reduced infection in barley, although the stimulating effect of light and heat obtained by growing non-vernalized plants in the glasshouse had no effect on the infection of wheat and barley.

There is, of course, variation in the amount of loose smut in crops between one season and another, depending on the weather conditions during the flowering period of the mother crop. The most important condition necessary for infection appears to be adequate humidity, and the disease was unusually troublesome in stocks of wheat and barley in this country following the wet summers of 1953 and 1954. This, however, is a different matter and does not concern the present discussion.

It is concluded, therefore, that the incidence of loose smut of wheat is not affected by external conditions at sowing time or thereafter during the growing period of the crop. Once an embryo of a susceptible variety becomes infected, the plant developing from that seed will be infected, and a proportion of the ears will be smutted on emergence.

We are grateful to Mr W. C. Stigwood of the Botany School, Cambridge, for preparing some of the sections.

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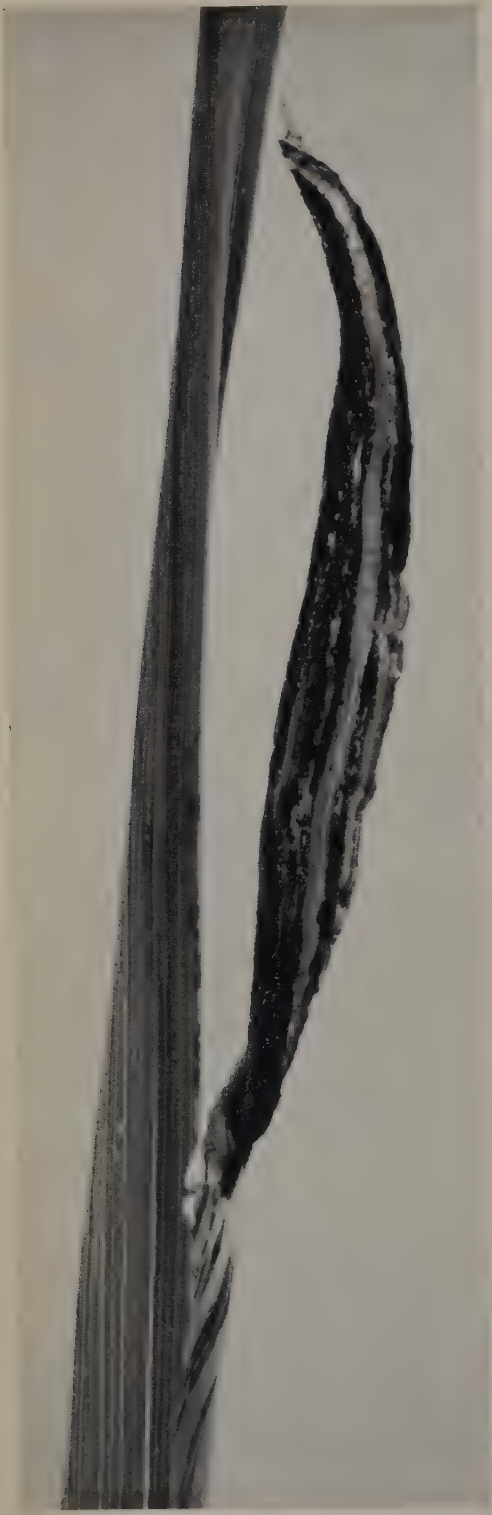

Fig. 1

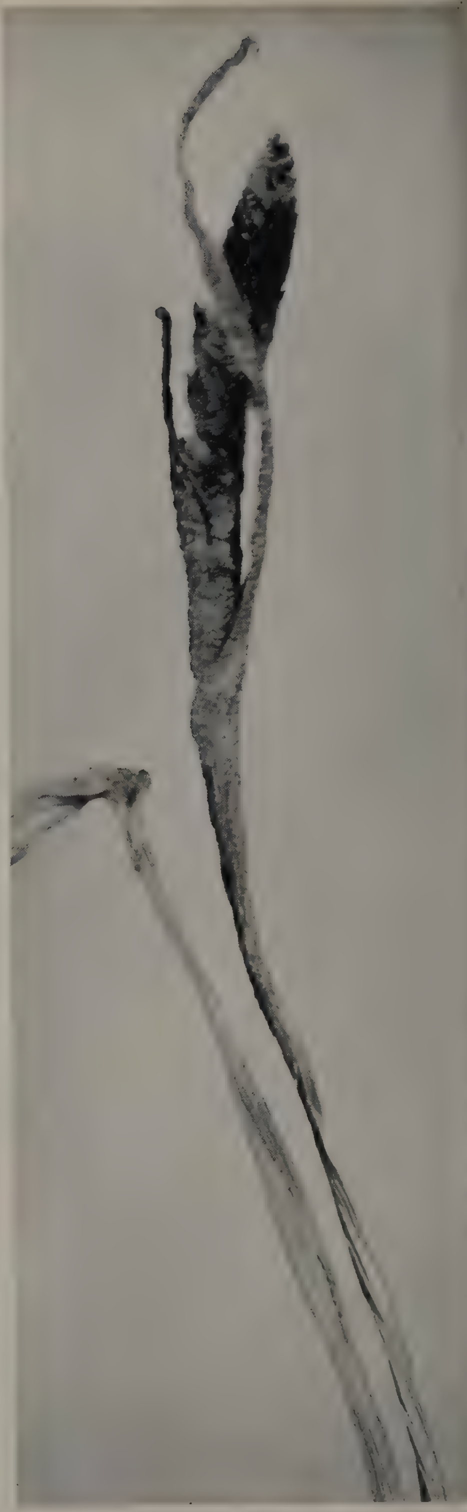

Fig. 2

(Facing p. 125)

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EXPLANATION OF PLATE 4

Ustilago tritici on wheat

Fig. 1. Spore masses on flag leaf and sheath of infected wheat tiller.

Fig. 2. Severe infection: flag leaf and sheath completely infected, with occasional stripes of spores on leaf below and its sheath.

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Trans. Brit. mycol. Soc. **41** (1), 126–128 (1958)

SCLEROPHOMA PITHYOPHILA ASSOCIATED WITH NEEDLE-CAST OF PINES AND ITS CONNEXION WITH *PULLULARIA PULLULANS*

By S. BATKO, J. S. MURRAY and T. R. PEACE

Forest Research Station, Alice Holt Lodge, Wrecclesham, Surrey

(With Plate 5)

Severe defoliation of Scots pine by the gall midge *Cecidomyia baeri* associated with the fungus *Sclerophoma pithyophila* is described. Culture work supported Robak's view that a form of *Pullularia pullulans* is a stage of *Sclerophoma*. *Phoma acicola*, quoted by several authorities as synonymous with *Sclerophoma pithyophila*, occurs under different pathological circumstances from *Sclerophoma* and is probably less important.

Sclerophoma pithyophila (Corda) v. Höhn. has been recorded fairly commonly as a saprophyte on dead pine needles, but has not until recently attracted attention as a possible pathogen in Britain. In Russia Nazarova (1936) has associated it with dieback and witch's broom formation in pine, while dieback of *Pinus contorta* has been attributed to it in Finland by Kujala (1948, 1950). He described the disease as affecting the tips of the leaders and upper branches. First the buds and the ends of the shoots died back, later the fungus spread downward through the pith. However, he considered that it was usually a saprophyte on shoots damaged by frost, drought, etc. Recently Jahnel & Junghans (1957) have associated this fungus with two apparently different diseases of Scots pine (*P. sylvestris* L.). In the first all the older needles were shed, and in the second those of the current year. In the second there was also a definite association of the fungus with damage caused by the pine gall midge, *Cecidomyia baeri* Prell.

Haddow (1941) found *Pullularia pullulans* (De Bary) Berkh. colonizing and extending the injury initiated by a Cecidomyid gall midge on the needles of red pine, *Pinus resinosa* Ait. His account of the damage caused by the combined activities of the insect and the fungus agrees very closely with that described below on Scots pine in Britain. Haddow, however, did not apparently observe any fruit-bodies of *Sclerophoma*. Patton & Riker (1954) also consistently isolated *Pullularia pullulans* from withered needles of *Pinus resinosa*; but they were unable to get any evidence of its pathogenicity and regarded it as purely secondary to drought injury.

In 1955 *Sclerophoma* was found locally in Norfolk associated with serious defoliation of the current year's needles of Scots pine. The symptoms were characteristic. The base of the needles collapsed, and became yellow, while the upper parts stayed green so that the needles hung upside down for some time before they fell. On severely affected shoots all needles might be attacked, so that complete defoliation took place, but frequently only some of the needles dropped, these being scattered among the healthy ones (Pl. 1, fig. 1). When the diseased needle fascicles fell they tended to hook on the remaining healthy ones and to remain hanging there. Dead needles hanging on the live ones in this way form the most striking symptom. Collapse of the needles took place in late summer, and the small black fruit bodies of *Sclerophoma* were produced in the autumn and winter (Pl. 1, fig. 2). Defoliation was most severe in the upper part of the tree. So far in Norfolk the disease has been found only on Scots pine 5–10 years old, 3–15 ft. in height.

Examination of diseased material by Mr D. Bevan, to whom our thanks are due, showed that a high percentage of the dead and dying needles had at the base the minute tunnels of *Cecidomyia baeri*. It seems probable therefore that in this instance the role of the fungus is to colonize the injuries already caused by the insect, and probably to extend the damage. Here our observations agree in general with those made by Haddow and also with those made by Jahnel & Junghans on their second *Sclerophoma* disease, except that they found that death of the needles started at the tip, not at the base.

Casual observation has also suggested that the fungus may be associated with a more normal type of needle-cast of Scots pine in Britain. This is the kind of injury, loss of older needles, that Jahnel & Junghans described for their first *Sclerophoma* disease.

Isolations from diseased material from Norfolk have repeatedly yielded *Pullularia* (*Dematium*) *pullulans*. This has previously been connected with *Sclerophoma pithyophila* (Robak, 1952). As a check, surface-sterilized pine needles collected from healthy trees were inserted in the cultures of *Pullularia* made from pine needles infected with *Sclerophoma*. In about 90 % of such tubes fruit-bodies of *S. pithyophila* developed on the needles, while no *Sclerophoma* developed on needles in control tubes containing malt agar.

In Britain, therefore, we appear to be concerned with an insect-fungus complex in which the pine gall midge, *S. pithyophila* and a form of *Pullularia pullulans*, which may well be stages of the same fungus, are involved. It seems probable, in agreement with the views expressed by Robak (1952), that we are here concerned with only one form of *P. pullulans*.

There is a possible connexion also between *Sclerophoma pithyophila* and *Phoma acicola*. von Höhnel (1909) first established the genus *Sclerophoma* and regarded *Phoma acicola* as synonymous with *Sclerophoma pithyophila*. Later, Diedicke (1915, 161, 280) described them as separate species, but in the same work (p. 897) quoted von Höhnel and followed him in referring to them as synonymous. Klebahn (1933) also, after a thorough anatomical and cytological investigation, considered them identical. Grove (1935, 157), apparently unaware of Klebahn's work, took the same view. This may well be true mycologically, but it would appear likely that pathological differences do exist. We have not found pycnidia agreeing with the original description of *Phoma acicola* on the collapsed needles in the disease described above. On the other hand, *P. acicola* has been far more widely found than pycnidia of the *Sclerophoma pithyophila* type on both Scots and Corsican pines. *Phoma acicola* is one of the commonest fungi on the dead or dying needles of Corsican pine (*Pinus nigra calabrica* Schneid.), the branches of which are dying back, owing to unsuitable climatic conditions aided by *Crumenula pinea* (Karst.) Ferd. & Jorg.

Further work is certainly called for on the pathogenicity of *Sclerophoma pithyophila*, *Pullularia pullulans* and *Phoma acicola*, and of the possible connexion between them. In the meantime the authors would be interested to hear of further instances of *Sclerophoma pithyophila* on prematurely dying pine needles, a condition which has so far been recorded in Norfolk, Suffolk, Dorset, Wiltshire, and Northumberland. The fungus has probably been recorded over a much wider area. The main interest is its association with actual disease.

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EXPLANATION OF PLATE 5

Fig. 1. Symptoms of *Sclerophoma pithyophila* and *Cecidomyia baeri* attack on Scots pine, showing the scattered distribution of the affected needles. Thetford Chase, Norfolk, December 1955.

Fig. 2. The fruit bodies of *Sclerophoma pithyophila* on Scots pine needles still attached to the tree. Thetford Chase, Norfolk, December 1955.

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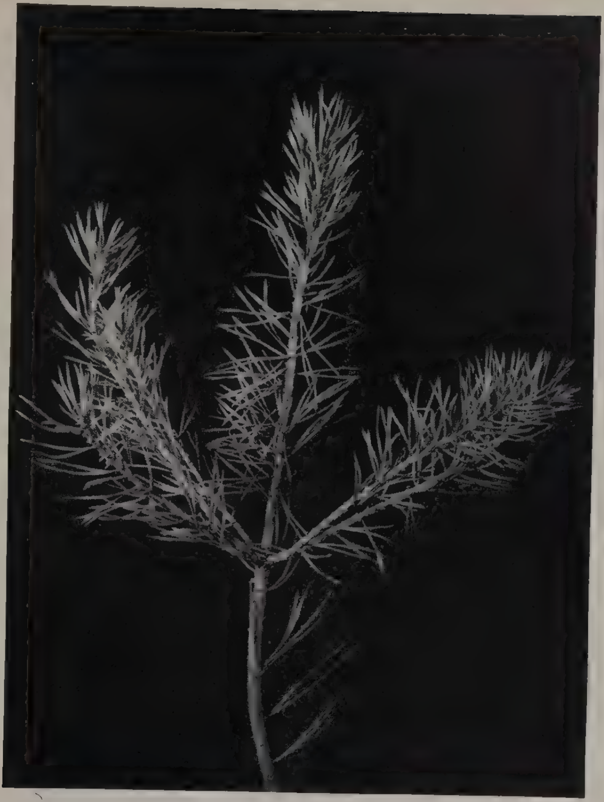


Fig. 1

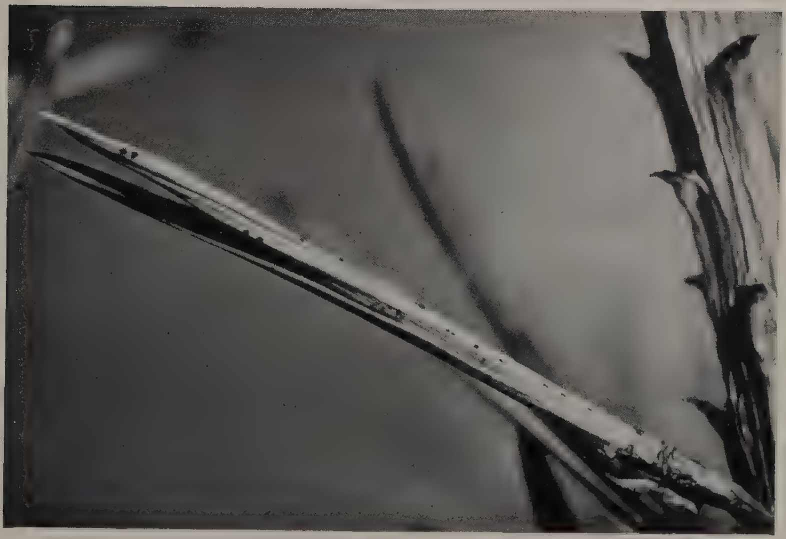


Fig. 2

Trans. Brit. mycol. Soc. **41** (1), 129–134 (1958).

THE THELEPHORACEAE OF THE MUSSOORIE HILLS (INDIA). II

By DEREK A. REID
Royal Botanic Gardens, Kew, England
K. S. THIND and K. L. ADLAKHA
Botany Department, Panjab University, Amritsar, India

(With Plate 6 and 3 Text-figures)

Descriptions are given of *Stereum sparassoides* (P. Henn.) comb.nov., previously known only from the type collection, and of *Craterellus mussooriensis* sp.nov., *C. cymatodermoides* sp.nov., and *Hymenochaete fusco-badia* sp.nov.

Under the leadership of Prof. P. N. Mehra an excursion is made every year from the Botany Department of the Panjab University to the Mussoorie Hills in the north-western Himalayas (2000–7000 ft. altitude) to study the cryptogamic flora. The Thelephoraceae are being investigated by Dr K. S. Thind and his students.

In the first contribution (Thind & Adlakha, 1957) eight species and one new variety were described. This paper deals with three new, and one little known, species. The fruit-bodies have been described from fresh specimens, supplemented with dried material and that preserved in alcohol formalin.

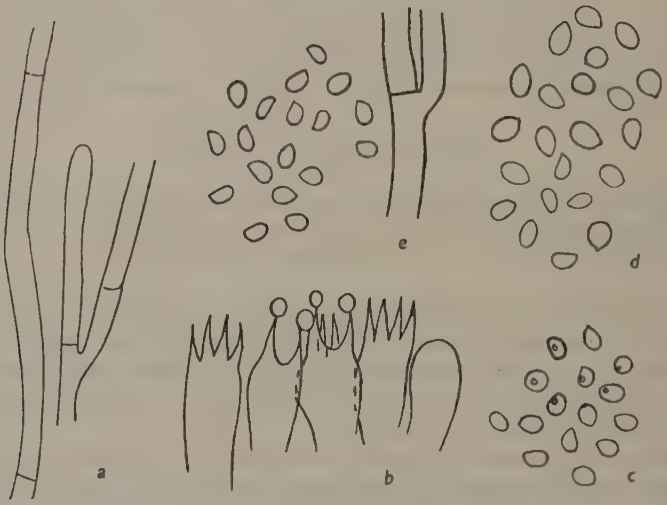
Type material of the new species has been deposited in the herbaria of the Panjab University and the Royal Botanic Gardens, Kew.

The numbers of the species are the serial numbers of the Thelephoroid flora.

9. **Stereum sparassoides** (P. Henn.) comb.nov. (Pl. 6, fig. 1; Text-fig. 1).

Synonym: *Thelephora sparassoides* P. Henn., in *Hedwigia*, **40**, 324, 1901.

Sporophore stipitate, coriaceous, 1–4 cm. in total height, 1·5–2·5 cm. diam., flabelliform or infundibuliform, adjacent fruit-bodies frequently confluent. *Pileus* pale brown, profusely lobed especially when mature, and marked by radiating ridges and grooves, appearing almost radiately plicate and obscurely concentrically sulcate in spirit material, margin incised, crenulate, lighter coloured. *Hymenium* decurrent, violet-grey, more or less smooth in dried specimens, but appearing uneven to radiately ridged in spirit material especially towards the margin. *Stipe* lateral or central, dark brown, solid, glabrous, usually short and flattened, sometimes cylindrical, 0·5–2 × 0·1–0·2 cm. *Basidia* clavate, subhyaline, 31·2–44·2 × 4–4·5 μ, with 2–4 straight sterigmata 4–6 μ in length. When mature the basidia, many of which are 2-spored, protrude above the hymenial surface. *Spores* hyaline, smooth, very broadly elliptical to subglobose, non-amyloid, 3·5–4·5 × 2·5–3 μ, with a very small highly refractive guttule which is variable in position. The spores of this species are rather variable, tending to be more globose on some fruit-bodies than on others, whilst in spirit material they are slightly larger, 4·5–5·5 × 2–4 μ, and lack the guttule. *Structure* monomitic, consisting of thin-walled, branched, septate, hyaline hyphae (appearing light brown in the mass), 2–4 μ wide, lacking clamp connexions.



Text-fig. 1. *Stereum sparassoides*: (*a*) hyphae, × 1000; (*b*) basidia, × 1530; (*c*) spores from dried material, × 866; (*d*) spores from spirit material, × 866; (*e*) spores and hypha from the type collection, × 866.

Habitat growing mostly in caespitose clusters, forming small rosettes, but sometimes solitary, on soil or on decaying twigs embedded in the soil, Dhobi Ghat, Mussoorie, India, 23 August 1954, No. 192.

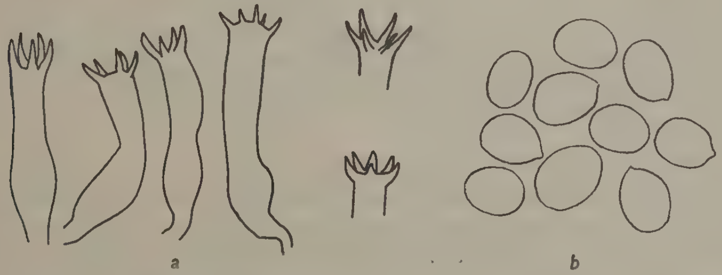
Hitherto *S. sparassoides* was known only from the type gathering, details of which are as follows: Arnigadh, Mussoorie, 5000 ft., coll. W. Gollan, 10 September 1901, on dead branches. Presumably Hennings's original material was lost in the destruction of the Berlin Herbarium during the last war, but a few fragments are preserved in the Herbarium of the United States Department of Agriculture. Whilst these are insufficient to give a clear idea of the macroscopic appearance of the fungus, it is possible to examine the microscopic structure. These fragments bear minute, hyaline, broadly elliptical spores, 3·5–4·5 × 2–3 μ, although Hennings found them to be subovoid, hyaline, smooth, 2–4 μ, and to have a monomitic context formed of thin-walled, hyaline, twisted ribbon-like, branched, septate hyphae, 3–5 μ diam., lacking clamp connexions. Hennings's original description of the species was as follows: 'Caulicola, membranaceo-papyracea, confluens, cristata; pileo substipitato, flabellato, plicato, fisso, margine palmato-dentato, dentis saepe subulatis, cremeo, 5–7 mm. longo, 2–3 mm. lato; hymenio plicato-pallido.' He added that it was a dainty little species resembling a *Sparassis* and related to *Thelephora tuberaoensis* P. Henn.

S. sparassoides is extremely closely related to *S. burtianum* Peck, which was described from the United States, differing only in the colour of the hymenium and slightly smaller basidia. In *S. sparassoides* the hymenium of fresh specimens is violet-grey, whilst in *S. burtianum* it is pale bay or ochraceous bay (Saccardo, 1905) or yellow ochre to pinard yellow (Burt, 1920). The spores of dried material of both species are very similar in size and shape, although very slightly larger in *S. burtianum* (3·5–5 × 2·5–3·5 μ).

10. **Craterellus cymatodermoides** sp.nov. (Pl. 6, fig. 2; Text-fig. 2)

Sporophora stipitata, 2·5–3·6 cm. alta, 0·7–2·2 cm. diametro. *Pileus* umbilicatus, in vivo fusco-violaceus, cristis radiato-acutis ornatus. *Hymenium* griseo-albidum, costis ornatum. *Stipes* centralis, hirsutus, griseo-albidus 1·5–3 cm. longus, 0·1–0·2 cm. latus. *Basidia* tetra- vel hexaspora cylindrica vel subampullacea, pallide straminea, 39–50 × 7–8 μ. *Sterigmata*, usque ad 8 μ longa, incurvata. *Sporae* hyalinae vel pallide straminiae, laeves, nonamyloideae (6–) 7–8 × 5–5·5 μ. *Hyphae* 3–9 μ diametro, ramosae, septatae, sine fibulis; muris pallide stramineis tenuibus.

Sporophores centrally stipitate, 2·5–3·6 cm. in total height, 0·7–2·2 cm. diam., umbilicate, slightly lobed, dark violet above when fresh, with radiating knife-edged crests corresponding to the venation of the hymenium. The greyish white hymenial surface is thrown into a complex system of branching folds giving a venose appearance. This consists of a number of prominent radiating primary ribs running out from the stipe to the margin, and from them arise, at acute angles, densely crowded narrower pinnate secondary ribs. The pinnate ribs from adjacent main ribs become fused together, except towards the margin of the pileus where they run out freely. Each primary rib with its system of pinnate secondary ribs resembles the venation of certain angiosperm leaves, whilst the hymenial configuration as a whole is very like that of some species of *Cymatoderma*. The greyish white solid stipe, 1·5–3 × 0·1–0·2 cm., is profusely hairy and expands above into the pileus. The 'hairs' on the stipe consist of conical aggregations of pale straw-coloured, branching, septate hyphae, which are up to 9 μ wide at the base, but with the apices and ultimate



Text-fig. 2. *Craterellus cymatodermoides*: (*a*) basidia, ×650; (*b*) spores, ×1150.

branches only 2·5 μ diam. *Basidia* cylindrical to suburniform, pale straw-coloured, 39–50 × 7–8 μ with 4–6 curved sterigmata up to 8 μ in length. When mature the basidia project up to 16 μ above the hymenial surface. *Spores* hyaline to very pale straw-coloured, non-amyloid, broadly elliptical to subglobose, with distinct lateral apiculus (6-)7–8 × 5–5·5 μ. *Cystidia* and *Gloeocystidia* absent. *Structure* monomitic, consisting of thin-walled, branched, septate, pale straw-coloured generative hyphae, 3–9 μ diam., lacking clamp connexions. The septa occur at intervals of 18–46 μ along the length of the hyphae, which are markedly constricted at these points. Radial sections through the fruit-body show a trama formed of dense horizontally orientated hyphae and a narrow subhymenial zone in which the hyphae are loosely and more or less vertically arranged. There is no distinct cuticular layer, and no evidence of a thickening hymenium.

Habitat growing singly or in caespitose clusters of two to three fruit-bodies on naked soil along the very recently eroded slopes of hills, Chakrata Toll, Mussoorie, India, 4 September 1954, No. 193.

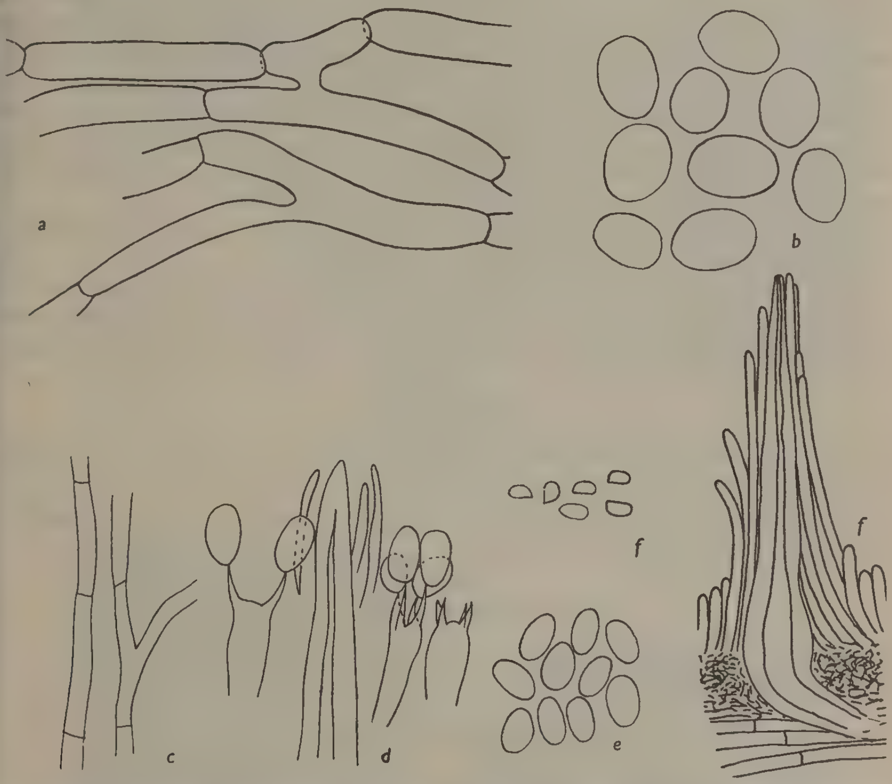
The species is well characterized by its peculiar hymenial configuration. It is common but not abundant. Occasionally a sporophore may proliferate and produce a second small fruit-body within the depressed central area of the pileus.

11. **Craterellus mussooriensis** sp.nov. (Pl. 6, fig. 3; Text-fig. 3)

Sporophora stipitata, 1·5–4 cm. alta, 0·7–2·2 cm. diametro. *Pileus* saepius orbiculatus, supra stipitem plus minusve depressus, pallide brunneus sinuosus; margine undulato dentato. *Hymenium* ochroleucum aliquando griseo-albidum, costis radiatis ornatum. *Stipes* saepius excentricus vel lateralis, nonnunquam centralis, solidus glaber, 0·8–2·5 cm. longus. *Basidia* tetraspora, clavata, hyalina, usque ad 67·6(–78) × 5–8 μ. *Sporae* hyalinae, laeves, nonamyloideae, late ellipsoideae vel subglobosae, 8–10·5(–11·5) × 6–8 μ. *Hyphae* 3–16 μ diametro, ramosae, septatae, sine fibulis, muris subhyalinis tenuibus, granulis brunneis incrustatae.

Sporophore stipitate, 1·5–4 cm. in total height, 0·7–2·2 cm. diam. *Pileus* usually circular, somewhat depressed opposite the point of attachment of the stipe, light brown, often conspicuously folded, and with dentate wavy margin. The folds of the pileus may become intimately fused one with another or with the stipe, the apical portion of which may then become enclosed within a pouch-like sac. *Hymenium* cream coloured but sometimes whitish grey, slightly ridged. These irregular ridges which radiate from the point of attachment of the stipe do not reach the periphery. *Stipe* usually excentric or lateral, rarely central, concolorous with the pileus, solid, cylindrical, glabrous, straight or slightly flexuous, 0·8–2·5 cm. long. *Basidia* clavate, hyaline up to 67·6 (–78) × 5–8 μ, with four incurved sterigmata. *Spores* hyaline, non-amyloid, smooth, aguttate, broadly elliptical to subglobose, 8–10·5(–11·5) × 6–8 μ, with granular contents when fresh. *Cystidia* and *Gloeocystidia* absent. *Structure* monomitic, consisting of thin-walled, branched, subhyaline hyphae, 3–16 μ diam., lacking clamp connexions. These generative hyphae, which may anastomose to form H-connexions, are constricted at the septa (these occur at intervals of 26–70 μ), and frequently bear a brown incrusting granular pigment. This pigment is very conspicuous in sections through the sporophore, where it often forms small brown granular areas. There is no distinct cuticular layer and no evidence of a thickening hymenium.

Habitat mostly gregarious or sometimes scattered, on soil along newly eroded hill slopes, Chakrata Toll, Mussoorie, India, 2 September 1953, No. 194.



Text-fig. 3. *Craterellus mussooriensis*: (*a*) hyphae, × 750; (*b*) spores, × 1150; *Hymenochaete fusco-badia*: (*c*) hyphae, × 750; (*d*) basidia and seta, × 1150; (*e*) spores, × 1150; (*f*) seta and spores from the Kew material, × 650.

12. **Hymenochaete fusco-badia** Thind & Adlakha, sp.nov. (Pl. 6, fig. 4; Text-fig. 3)

Sporophora resupinata, usque ad pedalia longa, rimosa. *Hymenium* fusco-brunneum vel atropurpureum; margine plus minusve albido. *Contextus* ferrugineus, 450–500 μ crassus. *Basidia* clavata, pallide brunnea, 3–4 μ crassa. *Sporae* subhyalinae, ellipsoideae, laeves, 7–8 × 4–5·5 μ. *Setae* fusco-brunneae, subulatae (30–)50–101 × (5–)8–10 μ. *Hyphae* 3–4 μ, crassae, flavo-brunneae, ramosae, septatae, sine fibulis, muris tenuibus.

Sporophore forming resupinate patches up to 1 ft. in length, abundantly cracked, coriaceous but becoming hard when dried, often separating from the substrate and enrolled at the margin. *Hymenium* deep brown or chocolate coloured, fading slightly on drying, rough under a lens due to the presence of setae; margin byssoid whitish. *Context* ferruginous, 450–500 μ thick. *Basidia* clavate, light brown, 3–4 μ wide. *Sterigmata* 4, straight, 6–8 μ long. *Spores* subhyaline, ellipsoid, smooth, thin-walled, 7–8 × 4–5·5 μ [hyaline elliptical, 4·5–6 × 2–3 μ in the Kew material, but very scanty]. *Setae* abundant, dark brown, subulate, thick-walled, arising in the context and forming only a single layer (30–)50–101 × (5–)8–10 μ, projecting 18–24 μ beyond the hymenial layer, and surrounded by pyramidal groups of very narrow, thin-walled hyaline hyphae. *Structure* monomitic, consisting of thin-walled yellowish brown, branched septate hyphae 3–4 μ wide, lacking clamp connexions. Sections through the sporophore show a context formed of rather loosely arranged hyphae and a narrow subhymenial zone, 15 μ wide, in which the hyphae are very densely compacted. This latter zone appears as a dark band beneath the hymenium, especially in older portions of the fruit-body.

Habitat on stumps and exposed roots of *Quercus incana*, Dhobi Ghat, Mussoorie, India, 23 August 1954, No. 195.

Collection No. 195 is closely allied to *H. semistupposa* Petch. However, it possesses only a monomitic hyphal system, and the much less abundant setae, which are not usually embedded, project above the hymenial surface.

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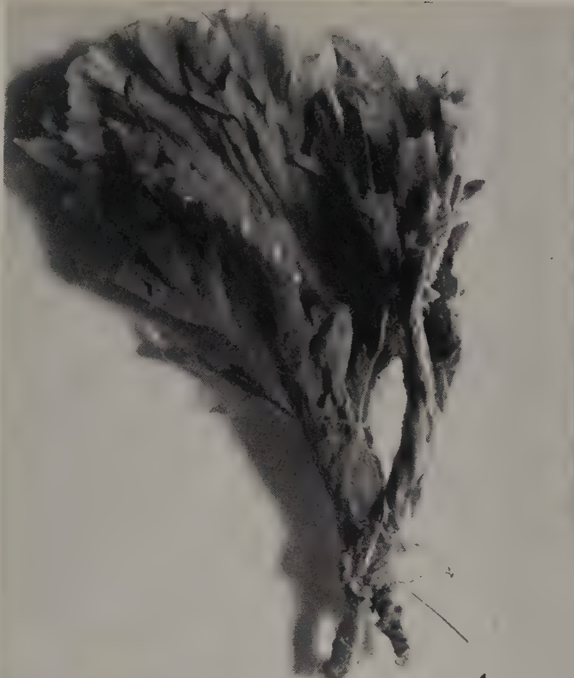
EXPLANATION OF PLATE 6

Fig. 1. *Stereum sparassoides*.
Fig. 2. *Craterellus cymatodermoides*.
Fig. 3. *Craterellus mussooriensis*.
Fig. 4. *Hymenochaete fusco-badia*.

(*Accepted for publication* 26 *July* 1957)



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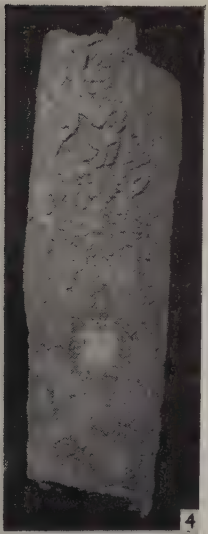

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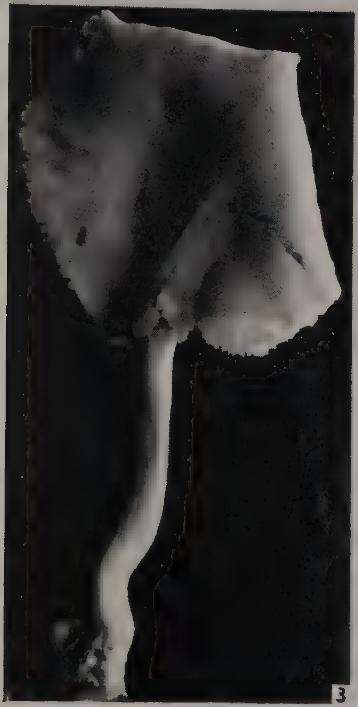

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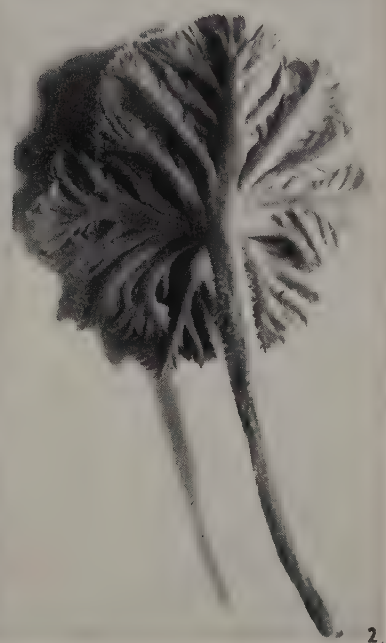

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Trans. Brit. mycol. Soc. **41** (1), 135–141 (1958).

TAXONOMIC NOTES ON THE GENERA *HELICOSTYLUM* AND *CHAETOSTYLUM* (MUCORACEAE)

By J. N. LYTHGOE
Botany School, University of Cambridge

(With 3 Text-figures)

Chaetostylum van Tiegh. & le Monnier is reduced to *Helicostylum* Corda. *H. venustellum* Lythgoe is proposed as a new species and *H. fresenii* (van Tiegh. & le Monnier) Lythgoe as a new combination. The terminal sporangium has a *Mucor*-type of columella, except in *H. piriforme* Bain. with a *Rhizopus*-type. A hemispherical columella is shown to occur in the sporangioles of *H. elegans* Corda and seems to occur also in *H. venustellum.* It is not apparent in *H. fresenii. H. piriforme* Bain. has pedicels growing in pairs on short dichotomously forked branches.

The systematic differences between the genera *Helicostylum* and *Chaetostylum* have long been uncertain. Two recent collections of fungi belonging to this group have enabled a fresh study of the subject to be made.

Helicostylum was erected by Corda (1842) for mucoraceous fungi resembling *Thamnidium elegans* Link but with circinate pedicels and no dichotomous branching. *H. elegans* Corda is the type species.

Chaetostylum was erected by van Tieghem & Le Monnier (1873) for fungi resembling *Thamnidium elegans* Link but with straight pedicels carried in verticils with no dichotomous branching. The type species is *C. fresenii* van Tieghem & le Monnier. (For synonymy, see Sumstine (1910), Hesseltine (1955, 1956).)

Bainier (1906) drew attention to a similarity between *H. elegans* and *C. fresenii*, following the discovery of *H. nigricans*, *H. glomeratum* and *H. piriforme* which had reflexed pedicels carried in umbels (Van Tieghem, 1876; Bainier, 1880). They differed from *H. elegans* in the columella of the primary sporangium, which is *Mucor*-like and not *Rhizopus*-like, and in the type of branching. Both these characters were considered similar in *C. fresenii* and *H. elegans*. Bainier therefore re-named *H. elegans*, *C. circinans*.

MATERIAL

In this study of *C. fresenii*, *H. elegans* and an intermediate taxon, the following material was used:

Chaetostylum fresenii: (1) F. T. Brooks, 1922. A typical strain of *C. fresenii* isolated from frozen meat. (Botany School, Cambridge, culture J.) (2) American Type Culture Collection, 11881. (3) Centraalbureau vor Schimmelcultures, Solberg.

Helicostylum elegans: (1) Corda, 1842, on rotton wood. Kew Herbarium. (2) Parfitt, 1871, on dead woodlice. Kew Herbarium. (3) Lythgoe, May

1955, on dead woodlice (strains A and L). (4) Lythgoe, April 1956, on dead woodlice a strain resembling L but not properly examined.

An intermediate form G (*Helicostylum venustellum*): With *H. elegans* in the April 1956 collection on dead woodlice.

Strains A, L and G have all been reduced to single spore cultures five times. They have retained their essential morphological characters since their original isolation.

Observations have also been made on *H. piriforme* (IMI 57446(a), 34453 and 52123), and on *Thamnidium elegans* Link (Botany School, Cambridge, culture M).

All descriptions are based on cultures on malt agar, a medium on which all the strains grew well.

TAXONOMIC CRITERIA

The following criteria have been used for separating the two genera in the literature; comments on these criteria based on observation of the cultured material have been added.

Type of branching

In *H. elegans*, the branches of the first order (Fig. 1 A) are not again branched, whilst in *C. fresenii* they are (Fig. 1 B, C) (van Tieghem & Le Monnier, 1873; Fitzpatrick, 1930; Naumov, 1939).

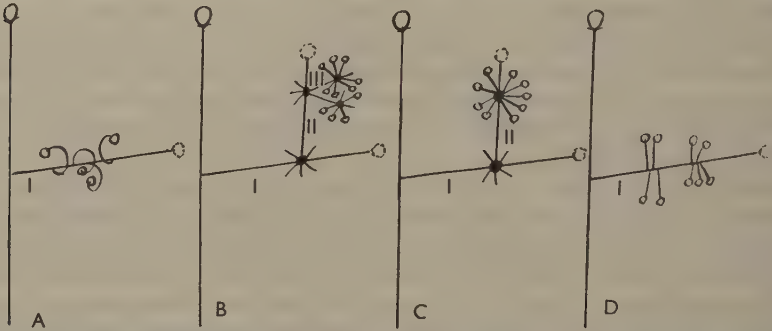

Fig. 1. Diagram of sporangiophores of: A, *H. elegans* Corda; B, C, *C. fresenii* van Tiegh. & le Monnier; D, *H. venustellum* Lythgoe (strain G). I, side branch of 1st order; II, side branch of 2nd order; III, side branch of 3rd order.

Brefeld (1891) described every degree of branching in *C. fresenii* (*Thamnidium chaetocladiodes* Bref.) from a condition where the pedicels are borne directly on the primary sporangiophore, to a complicated type where a third order of branches was present (Fig. 1 B); in my cultures, also, *C. fresenii* sometimes shows the third-order branches, although the usual second-order condition, as in Fig. 1 C, is more usual. In strains L and G the second-order branches are sometimes present also, and they are occasionally seen in strain A.

The verticillate arrangement of the pedicels in *C. fresenii* is very characteristic, and it is described and figured by most authors. It is typically present in my strains of *C. fresenii*. The pedicel arrangement described in the literature for *H. elegans* is more or less racemose (Fig. 1 A), and this arrangement is typically present in strains A and L; in strain G there is an arrangement, which although rather variable, might be described as a tight raceme (Fig. 1 D; Fig 3 A, B).

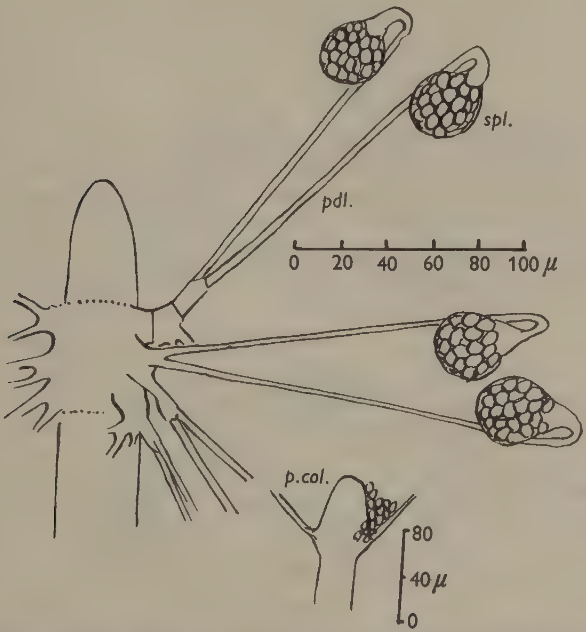

Fig. 2. *H. piriforme* Bain. *p.col.*, columella of primary sporangium; *pdl.*, pedicel; *spl.* sporangiole.

On the branches where the pedicels arise there are well-marked swellings in *C. fresenii* (Fig. 3C) (Sumstine, 1910; Hesseltine & Anderson, 1957), which are also shown in the illustrations of the fungus by other authors. There are no swellings described or figured for *H. elegans*. These swellings are present in my cultures of *C. fresenii* and appear before the pedicels become visible (Fig. 3C). The swellings in strain G are very slight and do not appear until the pedicel has emerged (Fig. 3B).

These swellings seem to be a reliable character, whilst the presence or absence of a second order of branching is a difficult character to see, and a variable one.

Form of the pedicels

The pedicels are straight in *H. elegans* and in *C. fresenii*. In my cultures, *C. fresenii* and strain G have straight pedicels and strains A and L have circinate ones. Strain A has very tightly coiled pedicels so that the sporangiole is in contact with the upper part of the pedicel, whilst the pedicels in L are not so tightly circinate.

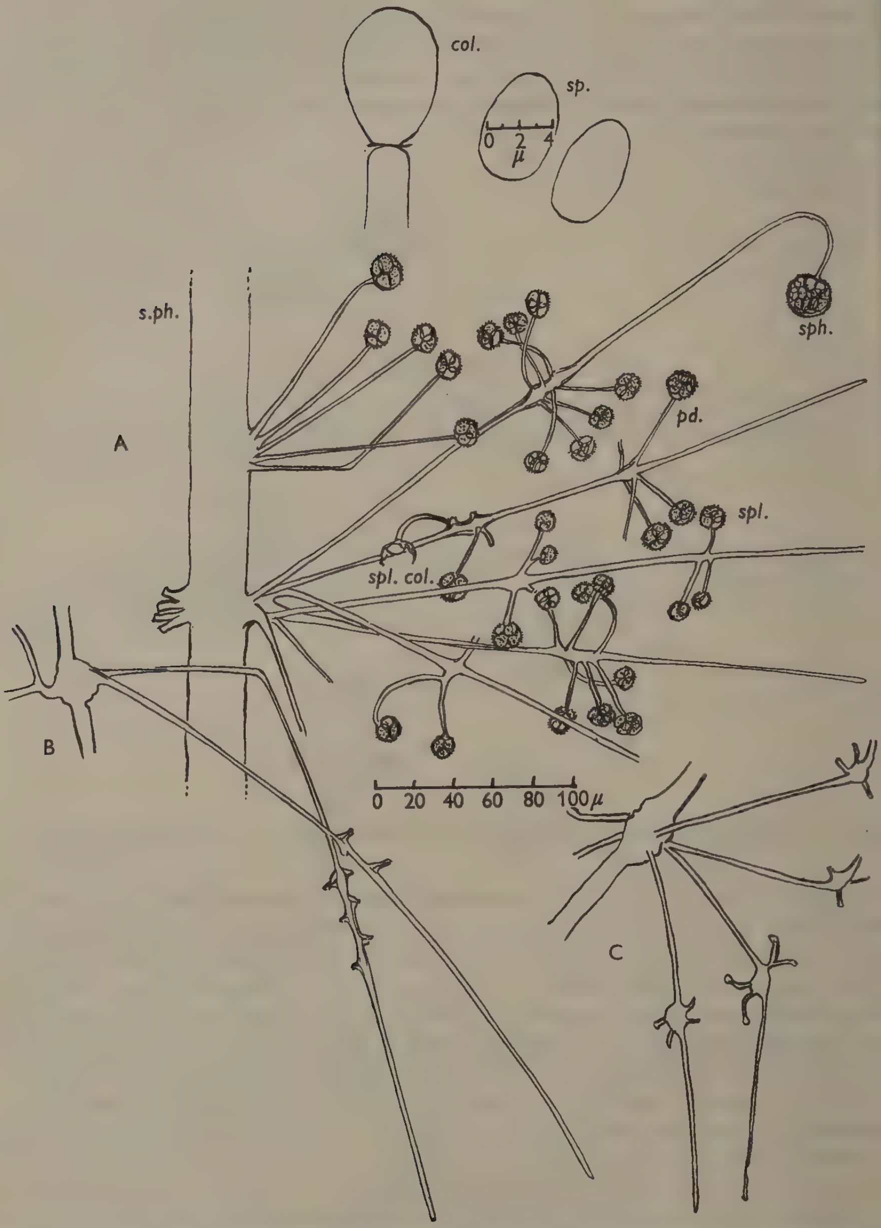

Fig. 3. A, *Helicostylum venustellum* Lythgoe; B, *H. venustellum*, young stage with no swellings on side branches; C, *H. fresenii* (van Tiegh. & le Monnier) Lythgoe at the same stage as B showing marked swellings on the side branches. *col.*, columella of primary sporangium; *pd.*, pedicel; *sp.*, spore; *sph.*, sporangiophore; *spl.*, sporangiole; *spl.col.*, sporangiole columella.

Columella of the sporangiole

Another interesting feature is the columella of the sporangiole. There is no agreement in the literature about the presence of the structure or on its morphological nature. Saccardo (1887), Corda (1842), Sumstine (1910) and Fitzpatrick (1930) say there is none. Zycha (1935) says it is much reduced in *Thamnidium* (sensu stricto), van Tieghem & le Monnier (1873) describe it as a very small domed septum which is often absent, Preus (1851) describes one for *Ascophora pulchra* Preus (*C. fresenii*, see Hesseltine & Anderson, 1957), but not for *T. amoenum* Preus (1852) (*H. elegans*).

Whole mounts of Parfitt's collection and strains G, A and L stained in neutral red or cotton blue showed a marked hemispherical columella.

Sections of strain A cut at 6 μ and stained in iron haemotoxylin or methylene blue and azure II show a distinct rift in the cytoplasm representing the columella before there is any sign of differentiation into spores. It is apparent that the columella, when it is present, is laid down in its final position, and is therefore a true columella and not a bulged septum. This columella is sometimes absent in small sporangioles.

CONCLUSIONS

Of the several characters used in taxonomic studies of these fungi, I have found the following to be reliable:

(1) The type of pedicel, whether it is straight, circinate or reflexed.

(2) The columella of the primary sporangium, either as in *Mucor* or *Rhizopus*.

(3) Swellings at the point of origin of the pedicels, either present or absent.

(4) Pedicel arrangement, either racemose or verticillate. In the true verticillate type the pedicels are borne on the swellings on the branches.

It is not considered that either the presence of sterile ends to the side branches, or whether these branches are themselves branched, are reliable characters.

On these characters, *H. elegans* has circinate pedicels, a *Mucor*-like primary sporangium, no swellings on the branches and racemosely arranged pedicels while *C. fresenii* has straight pedicels, a *Mucor*-like sporangium, and swellings on the branches which support verticils of pedicels. Strain G, an intermediate form between *Helicostylum* and *Chaetostylum*, has straight pedicels, a *Mucor*-like columella in the primary sporangium, no swellings on the branches, and more or less verticillately arranged pedicels.

Because of this intermediate form there seems to be no valid distinction between *Helicostylum* and *Chaetostylum* as represented by their type species. As *Helicostylum* is the older name it is suggested that *Chaetostylum fresenii* van Tieghem & le Monnier (1873, pp. 328–331) should be transferred to *Helicostylum* as **Helicostylum fresenii** (van Tiegh. & le Monn.) comb.nov.

H. elegans differs from other members of the genus *Helicostylum* as typified by *H. piriforme* in that

(1) The columella of the primary sporangium in *H. elegans* is *Mucor*-like, but it is *Rhizopus*-like in *H. piriforme*.

(2) The pedicels in *H. elegans* are circinate, whilst the sporangioles are reflexed on to the pedicels in *H. piriforme* (Fig. 2).

(3) *H. elegans* bears its pedicels singly in racemes (Fig. 1 A), whilst *H. piriforme* bears its pedicels in pairs on tight verticils or clumps of short dichotomous branches (Fig. 2).

Bainier's example is therefore followed in suggesting that *H. elegans* should be separated from *H. piriforme*.

Strain G appears to be distinct and is therefore proposed as a new species.

Helicostylum venustellum sp.nov. (Fig. 1 D, 3 A, B)

Hyphis sporangiferis erectis, 1–4 cm altis, sporangio magno terminatis vel lateraliter ramosis; ramis sine tumoribus, sporangila plurima pedicillata rectis gerentibus, apicibus sterilibus vel sporangiolo globoso, aliquando abortis, columella hemispherica praedito terminatis.

Sporangiis globosis, tunica fugacia, columella obovata; sporangiolis globosis asperis, saepe columella hemispherico; sporis ovideis in utroque sporangio aqualibus, hyalinis. 7·5–5·5 × 7·0–3·5 μ.

Habitat: In mortuis oniscis, Suffolk Anglia. Typus in Herb. Bot. School, Cantab. posuit.

Colony 1–4 cm. high. Primary sporangiophore erect with side branches borne in tight racemes or verticils; primary sporangium spherical, wall fugacious, columella obovoid. Side branches either sterile and straight, or terminated by a sporangiole perfect or abortive and slightly curved. Pedicels straight in verticils or tight racemes, not arising from pronounced swellings on the side branches, frequently also from the main sporangiophore. Sporangioles globose, spore-number 4–20 (typically 6), wall persistent, rough, a hemispherical columella usually present. Spores ovoid, hyaline, alike in both types of sporangium, 7·5 × 5·5–7·0 × 3·5 μ.

Habitat: Dead woodlice, April 1956, Suffolk, England. The type specimen has been deposited in the Herbarium of the Botany School, Cambridge, as no. 81.

I am indebted to Mr E. J. H. Corner, F.R.S., and to Dr N. F. Robertson for their great help in the preparation of this paper.

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(*Accepted for publication* 1 *June* 1957)

REVIEWS

Les Urédinées (*ou Rouilles des Végétaux*). Tome 3—*Genre* Uromyces: (*c*) Espèces parasites des plantes appartenant à la famille des *Légumineuses*. By A. L. GUYOT, 647 pp.; 143 text-figures, 13 tables. (*Encyclopédie Mycologique*, xxix.) (Paris, Paul Lechevalier: 14 April 1957.) Price Frs. 8500.

This volume continues Guyot's monographic treatment of *Uromyces* and deals with species with teleutospores on Leguminosae. These are grouped according to the ornamentation of the teleutospores. A list of host plants is given for each species, together with a detailed description, synonyms, and geographic distribution. References are made to the extensive bibliography given in *Les Urédinées*, Tome 1, and continued in *Uredineana*, Tomes 1–5.

Pages 582–621 give descriptions of aecidial forms (*Aecidium euphorbiae* Gmel., etc.) occurring on *Euphorbia*, which have been associated with *Uromyces* spp. on Leguminosae, and also of other species of *Aecidium* and *Endophyllum* described on *Euphorbia* which are morphologically similar to *Aecidium euphorbiae*.

The volume is well illustrated and indexed and should be welcome as a reference book to uredinologists.

F. C. DEIGHTON

Particulate Clouds: *Dusts, Smokes and Mists*. By H. L. GREEN and W. R. LANE. (London: E. and F. N. Spon, 1957.) Pp. xix + 425, 107 text-figures, 8 plates. Price: 70*s*.

The researches of Messrs H. L. Green and W. R. Lane at the Chemical Defence Experimental Station, Porton Down, bear on a variety of different aspects of applied science because, as they explain in the preface to this book: 'many different types of cloud have essentially the same properties'. The relevance of their topic to fungicidal dusts and sprays on the one hand, and to the dispersal of air-borne fungus spores on the other, explains why a book on the physics and physical chemistry of particulate clouds comes to be reviewed in the *Transactions*. Much of the first half of the book may not be easy reading, but the biologist is certain to be rewarded on consulting appropriate sections for information on such topics as sampling methods, dust removal, electrification of particles, evaporation of falling drops, deposition of particles on surfaces, and diffusion in turbulent air. The second half of the book deals with applications to industry and the environment; it includes health hazards due to air pollution and radioactive fall-out, natural clouds and artificial smoke screens, and pest control. We have long needed a survey of this field of ideas and Messrs Green and Lane have now provided it clearly and authoritatively in this excellent treatise.

P. H. GREGORY

Les Champignons d'Europe. BY ROGER HEIM. (Paris: N. Boubée et Cie, 1957. Vol. I, pp. 327, 24 pls. (4 cols.), 76 text-figures. Vol. II, pp. 572, 52 col. plates, 256 text-figures. Price: 7500 French francs (the 2 vols.).

The publication of yet another popular book on fungi is evidence, if such is needed, of the continued interest taken by the French public in the science of mycology. The first volume consists of a very comprehensive introduction to the study of fungi, followed by a descriptive section on the Ascomycetes. The second is devoted to the Basidiomycetes. Both volumes are well illustrated and the coloured plates are of a high standard. The use of line drawings to illustrate fungi is often very unsatisfactory, but in this instance the artist has succeeded in making them a praiseworthy feature.

This book, although far from being a complete flora, is intended not only for both amateur and professional mycologists, but also for those whose interest is mainly gastronomic. The introduction contains more detailed information than is usual in a work of this kind, and the novice will find the chapters on external morphology and microscopic structure particularly useful. Other topics include the interrelationships between the fungus and its environment, poisonous fungi and wood-rotting species.

The gastronome will find the chapters on cultivation of fungi, and cookery of special interest. There are thirty-nine recipes under the headings of 'Hors-D'Oeuvre et Salades', 'Potages', 'Fruits de Mer', 'Entrées', 'Œufs', 'Abats', 'Viandes' and 'Légumes'. In addition, there is a list of edible fungi divided into those species which are 'Succulents' (arranged in 'ordre de succulence décroissante'). 'Excellents', 'Agréables' (the species in the latter groups arranged in alphabetical order!), 'Assez Bons', and those 'Bons a l'État Cru'. It may come as something of a surprise to the growing band of British mycophagists to find that *Auricularia auricula-judae*, *Cantharellus cibarius* and *Peziza aurantia* come in the last category.

In the descriptive sections the author gives brief simplified descriptions of the species without microscopic data. He considers dichotomous keys to be unsatisfactory or dangerous for determination of *species*. However, there are numerous keys to the genera and the various groups of fungi under consideration. The Ascomycetes are dealt with very superficially, as is to be expected when so many species can only be identified with the aid of the microscope. However, the larger Discomycetes are adquately covered and the truffles are treated in some detail, but the Pyrenomycetes are dismissed in three pages. Of the Basidiomycetes, the Auriculariales, Tremellales, and Protoclavariales are briefly discussed, as are the less specialized members of the Aphyllophorales, but the Polypores and Clavarias are dealt with in greater detail. It is to the Boletales, Asterosporales (*Lactarius* and *Russula*) and Agricales that Prof. Heim devotes most attention.

Unfortunately the section of the book dealing with the latter groups shows some very regrettable features. The author makes it quite clear that he has little regard for the International Code of Botanical Nomenclature when he writes 'Les règles de la nomenclature, telles que les derniers Congrès internationaux les ont entérinées, sont à la fois le reflet d'une insuffisance de souplesse et d'un manque de logique, auxquels les esprits français, notamment, n'adhéreront jamais, du moins intégralement...'. On reading statements of this kind one cannot help wondering if the authors have given serious thought to the nomenclatural problems which would result if the Rules were made 'supple' and could be moulded to any individual's whims and fancies. It comes as a surprise to find that new genera are described in a work of this kind. The genus *Hodophilus* is proposed for two species which were previously placed in *Hygrophorus* or *Omphalia*, viz. *Hygrophorus foetens* and *H. atropunctus*, whilst the Friesian section *Hygrophani* of the subgenus *Pholiota* has been raised to generic rank as *Hygrophana* to accommodate *Pholiota mutabilis*. It is extremely difficult, whatever one's views on the Code, to understand the reasons which led Prof. Heim to propose a new genus for *P. mutabilis* when there is already an available genus—*Kuehneromyces*, with this fungus as the type species. The new subgenus *Constricta* is also proposed for *Tricholoma constrictum* and *T. leucocephalum*. One cannot help feeling that such important matters would have been better left to a separate scientific paper, and this applies to the description of the new species. These appear in the text with only brief descriptions lacking microscopic data, and the only indication that they are in fact new species appears in the index, where the name has a + against it. Even there some of the new species such as *Amanita malleata* are not indicated. Presumably the author intends to publish complete descriptions elsewhere, but as the species cannot be very common, it would have been better to omit all reference to them in this book. It should be noted that all the new names are nomina nuda; similarly all the new combinations made by the author, again without any indication, are illegitimate as they do not comply with the Code.

An example of the confusion which can result when the Code is ignored is seen in the case of *Psalliota terricola*. This fungus is marked as new in the index. It belongs to the *Xanthoderma* group in which Møller has described *P. meleagris* var. *terricolor*. Has Prof. Heim really described a new species or has he elevated a variety to specific rank?

In conclusion it can be stated that 'Les Champignons D'Europe' will, in the opinion of the reviewer, appeal more to the novice and to the mycophagist than to the professional mycologist. If they are willing to pay the rather high price, they will find this book both useful and interesting.

D. A. REID

Krankheiten und Schädlinge der Kulturpflanzen und ihre Bekämpfung. By H. Braun and E. Riehm. 8th, revised edition. (Berlin and Hamburg: Paul Parey, 1957.) 368 pp., 346 text-figures. Price: DM. 29.80.

The publication of a new edition of this work after only four years gives an indication of its popularity and of the authors' efforts to keep it up to date. The plan is unchanged, a concise introductory section being followed by chapters on individual crops, of which cereals, potato, fruit, and vine occupy the most space. The section on soybean is omitted, that on fruit now contains a brief review of virus diseases and an account of collar rot of apple, and some additional insect pests are considered. Illustrations are increased in number by 56. Some new literature is cited among the references; these might, however, be more conveniently assembled at the end of the book rather than in their present position as footnotes.

For the plant pathologist much of the value of this work probably lies in the fact that diseases and pests are treated together; thus the keys provided for each crop help one to distinguish at a glance between the symptoms caused by fungus and virus, bacterium and insect—a realistic approach. A wide field is covered. To what extent the coverage is adequate can best be decided by the practical use of the book; one notable omission, however, appears to be that of virus diseases from the section on cabbage.

D. JEAN STAMPS

G. Lindau and P. Sydow. Thesaurus literaturae mycologicae et lichenologicae. Supplementum 1911–1930. A–D. By R. Ciferri. (Pavia: Renzo Cortina, 1957.) Pp. iv+689. Price: 20,000 Italian lire (for the complete supplement).

Every mycologist must have had cause to be thankful for assistance obtained from the *Thesaurus* which gives a virtually complete list of mycological publications up to 1910 and a warm welcome for this supplement covering the next 20 years is assured. The Supplement is clearly a 'must' for all those who possess the original *Thesaurus* or the photo-offset reprint available from the Johnson Reprint Corporation, New York, but it will also be an invaluable source book for publications on systematic and applied aspects of mycology for the 20 years under review.

The Supplement is on the same lines as the *Thesaurus* and the first instalment includes 7167 citations. Two or three further volumes are promised this year to complete the work.

G. C. AINSWORTH

Welsh Flowering Plants. By H. A. Hyde and A. E. Wade. Second edition. Revised by A. E. Wade. (Cardiff: National Museum of Wales, 1957.) Pp. xi+209, 4 plates, 7 text-figures. Price 12*s*. 6*d*.

This attractively produced volume will be a most useful addition to the 'Foray Box' whenever the Society visits Wales. As an introduction to the check list (which is firmly based on specimens in the National Museum of Wales) there are sections on general aspects of the Welsh flora and on the botany of the Welsh counties.

G. C. A.

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CONTENTS

PAGE

Presidential Address. *Phytophthora*—Plant Destroyer. By C. J. Hickman. (With portrait) 1

The use of Cellophane in growth studies on soil fungi. By A. Kerr 14

Studies on *Leveillula taurica* (Lév.) Arn. and other powdery mildews. By M. A. Nour. (With 4 Text-figures) 17

The physiology of sporulation in *Penicillium griseofulvum* Dierckx. By A. G. Morton, D. J. F. England and Daphne A. Towler 39

Conidiobolus rhysosporus Dreschler in Great Britain. By Vivienne J. Dring. (With 1 Text-figure) 52

Observations on Gasteromycetes. VI. Three British species of *Nidularia* Fr. Section *Sorosia* Tul.: Ecology and distribution. By J. T. Palmer. (With 2 Text-figures) 55

British Records, 17–20 64

Studies in the development and cytology of *Puccinia penniseti* Zimm. By U. K. Kulkarni. (With 5 Text-figures) 65

Colonization of roots of *Pisum sativum* L. by fungi. By H. Stenton 74

Fungal mycelium in dune soils estimated by a modified impression slide technique. By Juliet C. Brown. (With 1 Text-figure) 81

Studies on *Puccinia polysora* Underw. I. The world distribution of forms of *P. polysora* By R. H. Cammack. (With 2 Text-figures) 89

Poria healeyi sp.nov. the causal fungus of yellow straw rot in Jarrah (*Eucalyptus marginata*) Sm. By N. E. M. Walters. (With Plate 1 and 2 Text-figures) . . 95

Physiologic specialization in *Rhynchosporium secalis*. By H. Owen. (With Plates 2 and 3 and 4 Text-figures) 99

Aquatic hyphomycetes from Uganda and Rhodesia. By C. T. Ingold. (With 5 Text-figures) 109

The development of loose smut (*Ustilago tritici*) in susceptible varieties of wheat, and some observations on field infection. By C. C. V. Batts and Ann Jeater. (With Plate 4 and 3 Text-figures) 115

Sclerophoma pithyophila associated with needle-cast of pines and its connexion with *Pullularia pullulans*. By S. Batko, J. S. Murray and T. R. Peace. (With Plate 5) 126

The Thelephoraceae of the Mussoorie Hills (India). II. By D. A. Reid, K. S. Thind and K. L. Adlakha. (With Plate 6 and 3 Text-figures) 129

Taxonomic notes on the genera *Helicostylum* and *Chaetostylum* (Mucoraceae). By J. N. Lythgoe. (With 3 Text-figures) 135

Reviews 142

INSTRUCTIONS TO AUTHORS

Contributors are asked to be clear and concise, and to use a recent issue of the *Transactions* as a guide to the arrangement of headings and sub-headings, explanations of plates and figures, references to the literature, and other details. Each paper should start with a short summary. Contributors are urged not to adopt long serial titles and sub-titles.

The standards adopted in the *Transactions* are those outlined in the Royal Society's pamphlet, *General Notes on the Preparation of Scientific Papers* (1950, The Royal Society, Burlington House, London, W. 1, price 2*s*. 6*d*.). An exception is that in the *Transactions* citations of references to literature, although based on the Harvard system, include the full titles of the papers and complete page numbers. Titles of journals are abbreviated in accordance with *A World List of Scientific Periodicals*, 1952.

The scientific names of genera and species are printed in italics and should be underlined in the typescript. The title of the paper, headings, and subheadings may be in capitals, but should not be underlined.

The attention of authors proposing new taxonomic groups is drawn to the need to give adequate descriptions and figures, and to designate the types as required by the *International Code of Botanical Nomenclature*. The description of a new species should be accompanied by a figure, and whenever possible the type specimen should be deposited in one of the national herbaria·

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